“A STUDY OF ASSOCIATION OF INFLAMMATORY BIOMARKERS (IL-18, hs-CRP) AND SERUM LIPIDS IN TYPE 2 DIABETES MELLITUS PATIENTS WITH OR WITHOUT DIABETIC NEPHROPATHY”

REG NO. BG0110008

Dissertation

Submitted to the
KLE University, Belgaum, Karnataka

In Partial Fulfillment
of the requirements for the degree of

M. D.

in

GENERAL MEDICINE

DEPARTMENT OF MEDICINE,
JAWAHARLAL NEHRU MEDICAL COLLEGE,
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ENDORSEMENT

This is to certify that the dissertation entitled **"A STUDY OF ASSOCIATION OF INFLAMMATORY BIOMARKERS (IL-18, hs-CRP) AND SERUM LIPIDS IN TYPE 2 DIABETES MELLITUS PATIENTS WITH OR WITHOUT DIABETIC NEPHROPATHY"** is a bonafide research work done by **CANDIDATE REG NO. BG0110008.**

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LIST OF ABBREVIATIONS USED

ACE	- Angiotensin Converting Enzyme
AD	- Anno Domini
ADA	- American diabetes association
AER	- Albumin Excretion Rate
AGEs	- advanced glycosylation end products
ANOVA	- Analysis Of Variance
ATP	- Adenosine triphosphate
B.C	- Before Christ
BMI	- Body mass index
BMI	- Body Mass Index
CAD	- Coronary artery disease
CDC	- Centers for Disease Control and Prevention
CHS	- Cardiovascular Heart Study
Cms	- Centimeters
CRP	- Pneumococcal Capsular antigen Reactive Protein
DCCT	- The Diabetes Control and Complications Trial
DKA	- Diabetic ketoacidosis
dl	- deciliters
DM	- Diabetes Mellitus
DNA	- Deoxy ribonucleic acid
eGFR	- Estimated glomerular filtration rate
ELISA	- Enzyme linked immuno sorbent assay
ESRD	- End-stage renal disease
FPG	- fasting plasma glucose

GDM	- gestational diabetes mellitus
GFR	- glomerular filtration rate
HbA1c	- Hemoglobin A1c
HDL	- High Density Lipoprotein
HHS	- Hyperglycemic hyperosmolar state
HL	- Hepatic Lipase
HNF	- Hepatocyte nuclear transcription factor
HOMA –IR	- Homeostasis model assessment - insulin resistance
hs-CRP	- highly sensitive C reactive protein
ICAM	- Inter cellular adhesion molecule
ICMR	- Indian Council of Medical Research
IDDM	- Insulin dependent diabetes mellitus
IDF	- International Diabetes Federation
IDL	- Intermediate Density Lipoprotein
IFG	- impaired fasting glucose
IFN	- Interferons
IGT	- impaired glucose tolerance
IL	- Inter leukin
IL-18	- Interleukin - 18
IPF	- insulin promoter factor
Kg	- kilo grams
Lbs	- pounds
LDL	- Low Density Lipoprotein
Lp(a)	- Lipoprotein a
LPL	- Lipoprotein lipase

M2	- metres square
MCP	- Monocyte Chemoattractant Protein
M-CSF	- Monocyte colony Stimulating Factor
MDRD	- Modification of Diet in Renal Disease
Mg	- milligrams
MI	- Myocardial Infarction
Mmol	- milli mole
MMP	- Matrix Metalloproteinase
MODY	- maturity onset diabetes of young
mts	- metres
NIDDM	- Non Insulin dependent diabetes mellitus
No	- Nitric oxide
NPH	- neutral protamine hagedorn
OR	- Odds Ratio
PAD	- Peripheral artery disease
PAI-1	- Plasminogen activator inhibitor-1
PDGF	- Platelet derived Growth Factor
Pg	- Pico grams
PKC	- Protein Kinase C
PODIS	- The prevalence of Diabetes in India Study
PZI	- Protamine insulin
RAS	- Renin Angiotensin System
SD	- Standard Deviation
sTNFR-2	- soluble tumour necrosis factor receptor 2
TG	- TriGlycerides

TGF -B	- Transforming Growth Factor Beta
U.S	- United States
UAE	- Urine Micro-Albumin Excretion Test
UKPDS	- The United Kingdom Prospective Diabetes Study
UTI	- Urinary Tract Infection
VCAM	- Vascular cell adhesion molecule
VEGF-A	- Vascular endothelial growth factor A
VLDL	- Very Low Density Lipoprotein
WHO	- world health organization

ABSTRACT

Background and objectives

Type 2 DM is now recognized as an inflammatory condition associated with insulin resistance and abnormal endothelial vascular reactivity. Dyslipidemia and inflammation may promote renal disease via mechanisms of vascular endothelial cell dysfunction in type 2 diabetes mellitus (DM). The present study was undertaken to find out the association of inflammatory biomarkers (IL-18, hs-CRP) and lipid profile in type 2 diabetes mellitus with or without diabetic nephropathy.

Methodology

The present one year cross sectional study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. A total of 100 patients with type diabetes mellitus were selected for the study. Based on the simple random sampling where every third patient who fulfilled the selection criteria was included in the study.

Results

In the present study, males outnumbered (64%) females (36%) with male to female ratio of 1.77:1. Most of the patients (38%) were aged between 61 and 70 years and mean age was 60.3 ± 9.43 years. All the subjects had an abnormal hs-CRP (100%) with a mean of 19.96 ± 9.59 mg/L and IL-18 was elevated in 71%. The mean IL-18 level was 260.89 ± 69.79 pg/mL. 60% of the patients had urinary excretion of albumin ranging from traces to >5 mg/dL. It was observed that 44% of the study population belonged to stage 1. The mean eGFR level was

89.31 ± 40.56 mL/min. The lipid abnormalities and inflammatory biomarkers (IL-18, hs-CRP) were found to be significantly high in patients with diabetic nephropathy.

Conclusion and interpretation

Findings of this study indicate an association of diabetic dyslipidemia and chronic inflammation with the pathogenesis of diabetic nephropathy.

Keywords

Diabetes mellitus; Diabetic nephropathy; High sensitivity C reactive protein; Inflammatory bio-markers; Interleukin-18;

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Chapter 1

Introduction

INTRODUCTION

Diabetes Mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.[1]

Diabetes mellitus (DM) is a chronic and potentially disabling disease which is reaching an epidemic proportion in many parts of the world. It is a major and growing threat to global public health. The biggest impact of the disease is on adults of working age; particularly in developing countries. The vast majority of cases of the diabetes fall into two broad categories: those having little or no endogenous insulin secretory capacity (IDDM or type 1 DM) and those who retain endogenous insulin secretory capacity but have a combination of resistance to insulin action and an inadequate compensatory insulin secretory response (NIDDM, or Type 2 DM).[1,2]

Centers for Disease Control and Prevention (CDC) report in 2011 estimated that nearly 26 million Americans have diabetes.[3] Type 2 diabetes mellitus (DM) accounts for more than 90% of the diabetic population world wide. Additionally, an estimated 79 million Americans have prediabetes. Rates of diabetes are increasing worldwide. The International Diabetes Federation predicts that the number of people living with diabetes will to rise from 366 million in 2011 to 552 million by 2030.[4] The top 10 countries in number of people with diabetes are currently India, China, the United States, Indonesia, Japan, Pakistan, Russia, Brazil, Italy, and Bangladesh. The prevalence of diabetes and its adverse

health effects have risen more rapidly in South Asia than in any other region of the world.[5]

Thirty years ago, the prevalence of diabetes in India based on the Indian Council of Medical Research (ICMR) multicentric survey[6] was around two percent in urban India and one percent in rural India. In just three decades, these prevalence rates have shot up to 12 to 16% in urban India and three to eight percent in rural India, in adults over 20 years of age. These represents a 600 to 800% increase in prevalence rates of diabetes something which is unparallel in any Western nation. Indeed, India is now referred to as the "Diabetic Capital" of the world.

Further, DM is associated with several complications. The complications of diabetes mellitus include retinopathy, nephropathy, and neuropathy (both peripheral and autonomic). The risk for atherosclerotic vascular disease is also increased in persons with DM. The risk for microvascular and neuropathic complications is related to both duration of diabetes and the severity of hyperglycemia; the increased risk for vascular disease actually antedates the onset of hyperglycemia to the degree associated with diabetes mellitus.[1]

Type 2 DM is now recognized as an inflammatory condition associated with insulin resistance and abnormal endothelial vascular reactivity. Several studies[7-9] have documented a positive association between dyslipidemia and inflammation and end-stage renal disease (ESRD) or advanced chronic kidney failure. Dyslipidemia and inflammation may promote renal disease via mechanisms of vascular endothelial cell dysfunction in type 2 diabetes mellitus

(DM). Several potentially modifiable lipid and inflammatory biomarkers are elevated in the setting of moderately decreased GFR in men with type 2 DM and may be the link between renal insufficiency and increased risk for cardiovascular events in this population.[10]

However, the relation between these biomarkers and mild or moderate renal dysfunction has not been well characterized, especially in type 2 diabetes mellitus (DM). Sparse data, however, are available on the relation of lipids and inflammatory biomarkers and glomerular filtration rate (GFR) in type 2 DM.

Even in the face of compelling evidence in favor of this theory, there are very few studies done in this area especially in India. Hence the present study was undertaken to find out the association of inflammatory biomarkers (IL-18, hs-CRP) and lipid profile in type 2 diabetes mellitus with or without diabetic nephropathy which may reveal new approaches to the prevention of progressive renal insufficiency.

Chapter 2

Objectives

OBJECTIVES

To find out the association of inflammatory biomarkers (IL-18, hs-CRP) and lipid profile in type 2 diabetes mellitus with or without diabetic nephropathy.

Chapter 3

Review of Literature

REVIEW OF LITERATURE

HISTORICAL REVIEW

Diabetes is perhaps as old as mankind. Cognizance of symptoms related to diabetes and recognition of the disorder was confined to a few geographic and cultural locations in the Ancient Era (up to 600 AD).

The knowledge acquired during this period was lost sight of and progress was tardy and indiscrete during the medieval period (600 to 1500 AD).

With the advent of modern age (1500 to 1758 AD) and its progression to renaissance and industrial revolution (1750 to 1850 AD), certain key features of diabetes were rediscovered and some new information was generated which stand out as landmarks in characterizing diabetes.

During the later decades of the 19th and first half of the 20th century, all round progress was achieved in the knowledge of pathology, predisposing factors, management, course and complications of diabetes mellitus. Growth of knowledge has been very fast in course of the second half of the last century (contemporary period) involving epidemiology, genetics, immunology and molecular biology which has led to accumulation of voluminous information on various aspects of this versatile disorder.[6,11]

Some key developments in scientific and clinical understanding of diabetes may be summarized as follows:

The earliest mention of diabetes like illness characterized by polyuria can be traced to Eqyptian Papyrus dating back to around 1550 B.C.[11]

- The sweet taste of diabetic urine was noted in the 5th and 6th century AD by the Indian physicians and in the 17th century by Thomas Willis. The term 'Diabetes mellitus', an allusion to the honeyed taste of urine, was first used in the late 18th century by John Rollo and others, to distinguish it from other polyuric states in which urine was tasteless.[11]

- In 1776, Matthew Dobson discovered that diabetic serum as well as urine contained sugar, and concluded that diabetes was a systemic condition rather than a disease of kidneys.[11]

- Claude Bernard made numerous discoveries in the field of metabolism and diabetes during the mid to late 19th century, describing the storage of glucose in the liver as glycogen and hyperglycemia in experimental animals.[11]

- In 1889, Oskar Minkowski and Josef Von Mering observed that total pancreatectomy produced diabetes in dogs.[11]

- In 1893, Edovard Laguesse named that pancreatic islets after Paul Langerhans, who had described them in 1869, and suggested that they produced a glucose lowering substance. This then hypothetical hormone was named 'insulin' by Jean de Meyer in 1909, over a decade before its discovery.[11]

- Various workers, including George Zueler (Germany) and Nicolas Paulesco (Romania), isolated active but impure hypoglycemic extracts from the pancreas during the first two decades of the 20th century; but toxic side effects precluded their formal testing in diabetic patients.[11]

- Insulin was discovered at the University of Toronto in 1921, through collaboration between Frederick G. Banting, Charles H. Best, James B. Collip and J.J.R. Macleod. Insulin was extracted from chilled pancreas in an acid / ethanol mixture; the extracts were found to lower blood glucose levels in pancreatectomized dogs and were first tested in a human diabetic in January 1922.[11]

- Major advances in the understanding of diabetes and metabolism have included:

    - The sequencing of insulin in 1955 by Frederick Sanger and elucidation of its three dimensional structure in 1969 by Dorothy Hodgkin.

    - The measurement of insulin concentration using the first radio immunoassay by Solomon Berson and Rosalyn Yalow in 1959.

    - The isolation of proinsulin in 1967 by Donal Steiner's group.

    - Identification of specific insulin receptors by Pierre Freychet and colleagues in 1971, and

    - The sequencing of the insulin receptor in 1985.

Landmarks in insulin discovery and development[6]

Year	Contribution	Discovery, development
1869	Paul Langerhans	Identified Islet cells
1889	Joseph Von Mehring and Oskar Minkowski	Identified pancreas as the origin of fatal diabetes mellitus
1908	George Ludwig Zeuler	Injected 'acomatrol' pancreatic extract into dying patient
1921	Paulesco	Pancreatin (Insulin)
1921	Banting and Best	Work started at the University of Toronto in the month of April
1922	Banting and Best	Insulin Isolation
1923	Nordisk Insulin Laboratory	Started production of Insulin
1926	Abel	Prepared the first crystalline insulin
1934	Svedberg	Molecular weight of insulin was determined
1936	Hagedorn (Novo Nordisk)	Development of the first protamine Insulin (PZI)
1946	Hagedorn (Novo Nordisk)	Development of the first prolonged acting Insulin-Neutral Protamine Hagedorn (NPH) or Isophane insulin
Year	**Contribution**	**Discovery, development**
1952	Hallas-Moller and Schlichtkrull	Development of the Lente series of Insulin
1955	Frederik Sanger	Elucidation on the structure of insulin and awarded with Nobel prize
1964	Novo Nordisk	Premixed insulin preparation were made available
1981	Jan Markussen and associates	First commercially available human insulin preparation using DNA technology
1996	Eli Lilly and company	First commercially introduced insulin analog, Lispro
2000	Novo Nordisk	Rapid- acting insulin analog- insulin aspart made available
2000	Aventis Pharmaceuticals	Marketing of long-lasting form of insulin – insulin Glargine
2003	Novo Nordisk	Detemir another long-acting insulin analogue introduced

Diabetes mellitus refer to a group of common metabolic disorder that shares the phenotype of hyperglycemia. Several distinct types of DM exist and are caused by a complex interaction of genetics and environmental factors. Depending on the etiology of the DM, factors contributing to hyperglycemia include reduce insulin secretion, decreased glucose utilization, and increased glucose production.[1,12]

The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system. In the United States, DM is the leading cause of end-stage renal disease (ESRD), non traumatic lower extremity amputations, and adult blindness. It also predisposes to cardiovascular diseases. With an increasing incidence worldwide, DM will be leading cause of morbidity and mortality for the foreseeable future.[1,6,12-14]

CLASSIFICATION OF DIABETES MELLITUS

DM is classified on the basis of the pathogenic process that leads to hyperglycemia, as opposed to earlier criteria such as age of onset or type of therapy. The two broad categories of DM are designated as[6]

- Type 1
- Type 2

Both types of diabetes are preceded by a phase of abnormal glucose homeostasis as the pathogenic processes progresses. Type 1 diabetes is the result of complete or near-total insulin deficiency. Type 2 DM is a heterogeneous group

of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production. Distinct genetic and metabolic defects in insulin action and/or secretion give rise to the common phenotype of hyperglycemia in type 2 DM and have important potential therapeutic implications now that pharmacologic agents are available to target specific metabolic derangements. Type 2 DM is preceded by a period of abnormal glucose homeostasis classified as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT).[6]

Spectrum of glucose homeostasis and diabetes mellitus[15]

Type of diabetes	**Normal glucose tolerance (NGT)**	**Impaired fasting glucose or impaired glucose tolerance**	**Hyperglycemia**		
			Diabetes mellitus		
			Not insulin required	**Insulin required for control**	**Insulin required for survival**
Type 1	←	—	—	—	→
Type 2	←	—	—	—	
Other Specific types	←	—	—	—	- - - →
Gestational diabetes	←	—	—	—	
Time (years)	←	—	—	—	→
FPG (mg/dl)	< 100	100-125		≥ 126	
2-h plasma glucose (mg/dl)	< 140	140 – 199		≥ 200	

Etiologic classification of diabetes mellitus[6]

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

A. Immune-mediated

B. Idiopathic

II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance)

III. Other specific types of diabetes

A. Genetic defects of β-cell function characterized by mutations in :

1. Hepatocyte nuclear transcription factor (HNF) 4α maturity onset diabetes of young (MODY) 1
2. Glucokinase (MODY 2)
3. HNF – 1α (MODY 3)
4. Insulin promoter factor (IPF) 1 (MODY 4)
5. HNF – 1β (MODY 5)
6. Neuro D1 (MODY 6)
7. Mitochondrial deoxyribo nucleic acid (DNA)
8. Sub units of adenosine triphosphate (ATP) – sensitive potassium channel.
9. Proinsulin or insulin conversion

B. Genetic defects in insulin action.

   1. Type A insulin resistance
   2. Leprechaunism
   3. Rabson-Mendenhall syndrome
   4. Lipodystrophy syndromes.

C. Diseases of the exocrine pancreas – pancreatitis, pancreatectomy, neoplasia, cystic fibrosis, hemochromatosis, fibrocalculouspancreatopathy.

D. Endocrinopathies – acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, hyperthyroidism, somatostatinoma, aldosteronoma

E. Drug or chemical induced – Vacor, pentamidine, nicotinic acid, glucocorticoids, thyroid hormone, diazoxide, beta-adrenergic agonists, thiazides, phenytoin, α– interferon, protease inhibitors, clozapine, beta blockers.

F. Infections – congenital rubella, cytomegalovirus, coxsackie.

G. Uncommon forms of immune-mediated diabetes – "stiff-man" syndrome, anti-insulin receptor antibodies.

H. Other genetic syndromes sometimes associated with diabetes – Down's syndrome, Klinefelter's syndrome, Turner's syndrome, Wolfram's syndrome, Friedreich's ataxia, Huntington's chorea, Laurence-Moon-Biedl syndrome, myotonic dystrophy, porphyria, Prader-Willi syndrome.

IV. Gestational diabetes mellitus (GDM)

EPIDEMIOLOGY

Epidemiology

Diabetes is fast becoming the epidemic of the 21st century. Type 2 diabetes, which is more prevalent (more than 90% of all diabetes cases) and the main driver of the diabetes epidemic, now affects 5.9% of the world's adult population with almost 80% of the total in developing countries.[16]

Nowhere is the diabetes epidemic more pronounced than in India as the World Health Organization (WHO) reports show that 32 million people had diabetes in the year 2000.[17] World Health Organization reported that, 346 million people worldwide have diabetes. In 2004, an estimated 3.4 million people died from consequences of high blood sugar. More than 80% of diabetes deaths occur in low- and middle-income countries. WHO projects that, diabetes deaths will double between 2005 and 2030.[17]

Race

The prevalence of type 2 diabetes mellitus varies widely among various racial and ethnic groups. Type 2 diabetes mellitus is becoming virtually pandemic in some groups of Native Americans and Hispanic people. The risk of retinopathy and nephropathy appears to be greater in blacks, Native Americans, and Hispanics.[18]

Sex

Type 2 DM is slightly more common in older women than men.[18]

Age

While type 2 diabetes mellitus traditionally has been thought to affect individuals older than 40 years, it is being recognized increasingly in younger persons, particularly in highly susceptible racial and ethnic groups and the obese. In some areas, more type 2 than type 1 diabetes mellitus is being diagnosed in prepubertal children, teenagers, and young adults. Virtually all cases of diabetes mellitus in older individuals are type 2.[18]

Indian scenario

India is in the midst of an ever-increasing epidemic of diabetes mellitus. Data on type 1 diabetes mellitus from our country is scant. Clinic based data from the mid sixties to the eighties reported the prevalence of childhood diabetes with onset below 15 years of age as being one to four percent of all the diabetic subjects attending clinics in different parts of the country.[6,12]

According to recent study also, almost 95% of childhood diabetes reportedly belongs to Type 1 DM. Early onset type 2 diabetes, MODY, fibrocalculous pancreatic diabetes and diabetes associated with genetic syndromes accounted for the remaining cases.[6]

Type 2 DM accounts for more than 90% of all patients with diabetes in India. According to WHO there were an estimated 19.4 million diabetes individuals in 1995, and this number is projected to increase in 80 million by 2030. The ICMR study (1972 to 1975) was the first systematic nationwide collaborative study on the prevalence of diabetes mellitus.[6,12]

The prevalence of diabetes was found to be 2.8% in rural and five percent in the urban population above the age of 40 years. The prevalence of Diabetes in India Study (PODIS) carried out in 77 centres recently reported a standardized prevalence rate for DM, in the total urban and rural population of 4.3, 5.9 and 2.7% respectively.[6]

Several epidemiological studies in migrant Indians and India itself show that, the population has a high genetic predisposition for diabetes, which is precipitated by environmental factors such as urbanization.[16] The prevalence of diabetes is four to six fold lower in rural areas, which is probably attributed to a conventional lifestyle which has beneficial effect on glucose tolerance (IGT). National Urban Diabetes Survey done in six cities, found age standardized prevalence rates of 12% for diabetes; with a slight male preponderance and 14% for impaired glucose tolerance. Subjects under the age of 40 years, had a prevalence of five percent for DM and 13% prevalence of impaired glucose tolerance.

The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025.[19] It is clear that in the last two decades, there has been a marked increase in the prevalence of diabetes among both urban as well as the rural Indians, with a suggestion that Southern India has seen the sharpest increase. Subsequent studies confirmed this high prevalence of diabetes in urban south India. Although in rural India the prevalence of diabetes is much lower than in the urban population, even here the prevalence rates are rapidly rising, though clearly more studies are needed. Variations in the prevalence rates of diabetes in

different urban populations of India are expected because of the large variation in the prevalence of cardiovascular risk factors in different regions and states. It is evident that there is a shift in age of onset to younger age groups, which is alarming and this could have adverse effects on the nation's economy. Hence, the early identification of at-risk individuals and appropriate intervention to increase physical activity, bring about changes in dietary habits could to a great extent help to prevent/ delay, the onset of diabetes and thus reduce the burden due to its associated complications in India.[16]

The world wide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 177 million in 2000. Based on current trends, more than 360 million individuals will have diabetes by the year 2030. Although the prevalence of both type 1 and type 2 DM is increasing world wide, the prevalence of type 2 DM is rising much more rapidly because of increasing obesity and reduced activity levels as countries become more industrialized. This is true in most countries, and 6 of the top 10 countries with the highest rates are in Asia. In the United States, the centre for Disease control and prevention (CDC) estimated that 20.8 million persons, or seven percent of the population, had diabetes in 2005 (30% of individuals with diabetes were undiagnosed).[6,12]

The prevalence is similar in men and women throughout most age ranges but is slightly greater in men more than 60 years. World wide estimates project that in 2030 the greatest number of individuals with diabetes will be 45 to 64 years of age.[12]

Causes for diabetic pandemic

The type 2 DM epidemic is tightly and consistently linked to that of obesity, both geographically and chronologically. Many factors like, urbanization and mechanization, together with globalized pattern of western pattern of lifestyle, together with poverty, lack of education and low socio-economic status and inner city deprivation are emerging as significant risk factors for DM. Lack of breast feeding, low birth weight is associated with insulin resistance and type 2 DM in adult life (especially in subjects who become obese) due to long term metabolic response during poor fetal nutrition.[20]

Obesity

Prevention of obesity, in women of child bearing age, is another primary goal because exposure to environment of a diabetic pregnancy places the fetus at increased risk for future onset diabetes. About 80% of patients are obviously obese at the time of diagnosis, usually with a central fat distribution in and around the abdominal cavity. In addition, many of those who are not traditionally obese, by weight criteria have increased percentage of fat predominantly distributed in the abdominal region. It is the most obvious target to prevent DM.

Body mass index (BMI)

Three key anthropometric measurements are important to evaluate the degree of obesity – weight, height and waist circumference. The BMI, calculated as weight (kg)/height $(m)^2$, or as weight (lbs)/height$(inches)^2$ x 703, is used to classify weight status and risk of disease. Body mass index, is used since it

provides an estimate of body fat and is related to risk of disease. Lower BMI thresholds for overweight and obesity have been proposed for the Asia-Pacific region since this population appears to be at risk at lower body weights for glucose and lipid abnormalities.[1]

Classification of weight status and risk of disease[21]

	BMI (Kg/m^2)	**Obesity Class**	**Risk of Disease**
Underweight	<18.5		
Healthy weight	18.5 – 24.9		
Overweight	25.0 – 29.9		Increased
Obesity	30.0 – 34.9	I	High
Obesity	35.0 – 39.9	II	High
Extreme Obesity	≥ 40	III	Extremely high

CRITERIA FOR THE DIAGNOSIS OF DIABETES MELLITUS[11,12]

- Symptoms of diabetes plus random blood glucose concentration more than 11.1 mmol/L (200 mg/dL)[a] *or*
- Fasting plasma glucose more than 7.0 mmol/L (126 mg/dL)[b] *or*
- Two-hour plasma glucose more than 11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test[c]

Note: In the absence of unequivocal hyperglycemia and acute metabolic decompensation, these criteria should be confirmed by repeat testing on a different day.

[a]Random is defined as without regard to time since the last meal.

[b]Fasting is defined as no caloric intake for at least 8 h.

[c]The test should be performed using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water; not recommended for routine clinical use.

SCREENING[1]

Widespread use of the fasting plasma glucose (FPG) as a screening test for type 2 DM is recommended because:

1. A large number of individuals who meet the current criteria for DM are asymptomatic and unaware that they have the disorder.

2. Epidemiologic studies suggest that type 2 DM may be present for up to a decade before diagnosis.

3. As many as 50% of individuals with type 2 DM have one or more diabetes-specific complications at the time of their diagnosis.

4. Treatment of type 2 DM may favorably alter the natural history of DM. The ADA recommends screening all individuals more than 45 years every three years and screening individuals at an earlier age if they are overweight [body mass index (BMI) more than 25 kg/m^2] and have one additional risk factor for diabetes. In contrast to type 2 DM, a long asymptomatic period of hyperglycemia is rare prior to the diagnosis of type 1 DM.[1,6]

PATHOGENESIS

Type 2 DM

Insulin resistance and abnormal insulin secretion are central to the development of type 2 DM. Although the primary defect is controversial, most studies support the view that insulin resistance precedes an insulin secretory defect but that diabetes develops only when insulin secretion becomes inadequate.

Genetic Considerations

Type 2 DM has a strong genetic component. The concordance of type 2 DM in identical twins is between 70 and 90%. Individuals with a parent with type 2 DM have an increased risk of diabetes; if both parents have type 2 DM, the risk approaches 40%.

Insulin resistance, as demonstrated by reduced glucose utilization in skeletal muscle, is present in many nondiabetic, first-degree relatives of individuals with type 2 DM. The disease is polygenic and multifactorial since in addition to genetic susceptibility, environmental factors (such as obesity, nutrition, and physical activity) modulate the phenotype. The genes that predispose to type 2 DM are incompletely identified, but recent genome-wide association studies have identified several genes that convey a relatively small risk for type 2 DM (relative risk of 1.1 to 1.5). Most prominent is a variant of the transcription factor 7 like 2 gene that has been associated with type 2 diabetes in several populations and with impaired glucose tolerance in one population at high risk for diabetes. Genetic polymorphisms associated with type 2 diabetes have also been found in the genes encoding the peroxisome proliferators-activated receptor-r, inward rectifying potassium channel expressed in beta cells, zinc transporter expressed in beta cells, IRS, and calpain 10. The mechanisms by which these genetic alterations increase the susceptibility to type 2 diabetes are not clear, but several are predicted to alter insulin secretion. Investigation using genome-wide scanning for polymorphisms associated with type 2 DM is ongoing.[1]

PATHOPHYSIOLOGY

Type 2 DM is characterized by impaired insulin secretion, insulin resistance, excessive hepatic glucose production, and abnormal fat metabolism. Obesity, particularly visceral or central (as evidenced by the hip-waist ratio), is very common in type 2 DM. In the early stages of the disorder, glucose tolerance

remains near-normal, despite insulin resistance, because the pancreatic beta cells compensate by increasing insulin output.

As insulin resistance and compensatory hyperinsulinemia progress, the pancreatic islets in certain individuals are unable to sustain the hyperinsulinemic state. IGT, characterized by elevations in postprandial glucose, then develops. A further decline in insulin secretion and an increase in hepatic glucose production lead to overt diabetes with fasting hyperglycemia. Ultimately, beta cell failure may ensue.[1]

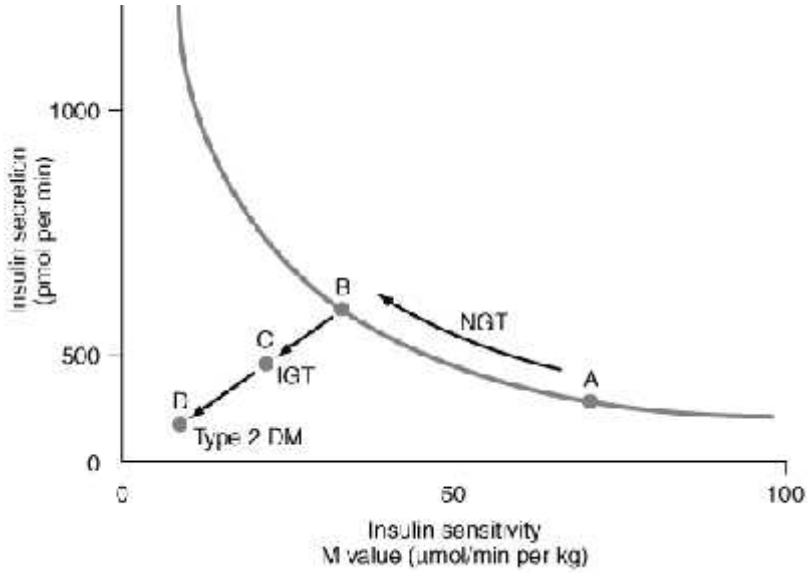


Figure 1. Metabolic changes during the development of type 2 diabetes mellitus[1]

COMPLICATIONS OF DIABETES MELLITUS[6,12]

Acute Complications of DM

Diabetic ketoacidosis (DKA) and hyperglycemic hyperosmolar state (HHS) are acute complications of diabetes. DKA was formerly considered a hallmark of type 1 DM, but it also occurs in individuals who lack immunologic features of type 1 DM and who can subsequently be treated with oral glucose-lowering agents (these obese individuals with type 2 DM are often of Hispanic or African-American descent). HHS is primarily seen in individuals with type 2 DM. Both disorders are associated with absolute or relative insulin deficiency, volume depletion, and acid-base abnormalities. DKA and HHS exist along a continuum of hyperglycemia, with or without ketosis. Both disorders are associated with potentially serious complications if not promptly diagnosed and treated.[6,12]

Chronic Complications of DM

The chronic complications of DM affect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease. Chronic complications can be divided into vascular and nonvascular complications. The vascular complications of DM are further subdivided into microvascular (retinopathy, neuropathy, nephropathy) and macrovascular complications [coronary artery disease (CAD), peripheral arterial disease (PAD), cerebrovascular disease]. Nonvascular complications include problems such as gastroparesis, infections, and skin changes. Long-standing diabetes may be

associated with hearing loss. Whether type 2 DM in elderly individuals is associated with impaired mental function is not clear.[12]

Chronic complications of diabetes mellitus

1. Microvascular
    a. Eye disease
        i. Retinopathy (nonproliferative/proliferative)
        ii. Macular edema
    b. Neuropathy
        i. Sensory and motor (mono- and polyneuropathy)
        ii. Autonomic
    c. Nephropathy
2. Macrovascular
    a. Coronary artery disease
    b. Peripheral arterial disease
    c. Cerebrovascular disease
3. Other
    a. Gastrointestinal (gastroparesis, diarrhea)
    b. Genitourinary (uropathy/sexual dysfunction)
    c. Dermatologic
    d. Infectious
    e. Cataracts
    f. Glaucoma
    g. Periodontal disease

The risk of chronic complications increases as a function of the duration of hyperglycemia; they usually become apparent in the second decade of hyperglycemia. Since type 2 DM often has a long asymptomatic period of hyperglycemia, many individuals with type 2 DM have complications at the time of diagnosis.[14]

The Microvascular complications of both type 1 and type 2 DM result from chronic hyperglycemia. Large, randomized clinical trials of individuals with type 1 or type 2 DM have conclusively demonstrated that a reduction in chronic hyperglycemia prevents or delays retinopathy, neuropathy and nephropathy. Other incompletely defined factors may modulate the development of complications.[22,23]

Mechanisms of Complications

Although chronic hyperglycemia is an important etiologic factor leading to complications of DM, the mechanism(s) by which it leads to such diverse cellular and organ dysfunction is unknown.[16]

Four prominent theories, which are not mutually exclusive, have been proposed to explain how hyperglycemia might lead to the chronic complications of DM.[1,22,25]

One theory is that increased intracellular glucose leads to the formation of advanced glycosylation end products (AGEs) via the nonenzymatic glycosylation of intra- and extracellular proteins. Nonenzymatic glycosylation results from the interaction of glucose with amino groups on proteins. AGEs have been shown to

cross-link proteins (for example collagen, extracellular matrix proteins), accelerate atherosclerosis, promote glomerular dysfunction, reduce nitric oxide synthesis, induce endothelial dysfunction, and alter extracellular matrix composition and structure. The serum level of AGEs correlates with the level of glycemia, and these products accumulate as glomerular filtration rate declines.

A second theory is based on the observation that hyperglycemia increases glucose metabolism via the sorbitol pathway. Intracellular glucose is predominantly metabolized by phosphorylation and subsequent glycolysis, but when increased, some glucose is converted to sorbitol by the enzyme aldose reductase. Increased sorbitol concentration alters redox potential, increases cellular osmolality, generates reactive oxygen species, and likely leads to other types of cellular dysfunction. However, testing of this theory in humans, using aldose reductase inhibitors, has not demonstrated significant beneficial effects on clinical endpoints of retinopathy, neuropathy, or nephropathy.

A third hypothesis proposes that hyperglycemia increases the formation of diacylglycerol leading to activation of protein kinase C (PKC). Among other actions, PKC alters the transcription of genes for fibronectin, type IV collagen, contractile proteins, and extracellular matrix proteins in endothelial cells and neurons. Inhibitors of PKC are being studied in clinical trials.

A fourth theory proposes that hyperglycemia increases the flux through the hexosamine pathway, which generates fructose-6-phosphate, a substrate for O-linked glycosylation and proteoglycan production. The hexosamine pathway may alter function by glycosylation of proteins such as endothelial nitric oxide

synthase or by changes in gene expression of transforming growth factor B (TGF-B) or plasminogen activator inhibitor-1 (PAI-1).

Growth factors appear to play an important role in DM-related complications, and their production is increased by most of these proposed pathways. Vascular endothelial growth factor A (VEGF-A) is increased locally in diabetic proliferative retinopathy and decreases after laser photocoagulation. TGF-B is increased in diabetic nephropathy and stimulates basement membrane production of collagen and fibronectin by mesangial cells. Other growth factors, such as platelet-derived growth factor, epidermal growth factor, insulin-like growth factor I, growth hormone, basic fibroblast growth factor and even insulin, have been suggested to play a role in DM-related complications. A possible unifying mechanism is that hyperglycemia leads to increased production of reactive oxygen species or superoxide in the mitochondria; these compounds may activate all four of the pathways described above. Although hyperglycemia serves as the initial trigger for complications of diabetes, it is still unknown whether the same pathophysiologic processes are operative in all complications or whether some pathways predominate in certain organs.[6]

Glycemic Control and Complications

The Diabetes Control and Complications Trial (DCCT) provided definitive proof that reduction in chronic hyperglycemia can prevent many of the early complications of type 1 DM. This large multicenter clinical trial randomized over 1400 individuals with type 1 DM to either intensive or conventional diabetes management, and prospectively evaluated the development

of retinopathy, nephropathy, and neuropathy. Individuals in the intensive diabetes management group received multiple administrations of insulin each day along with extensive educational, psychological, and medical support.[26]

Individuals in the conventional diabetes management group received twice-daily insulin injections and quarterly nutritional, educational, and clinical evaluation. The goal in the former group was normoglycemia; the goal in the latter group was prevention of symptoms of diabetes. Individuals in the intensive diabetes management group achieved a substantially lower hemoglobin A1C (7.3%) than individuals in the conventional diabetes management group (9.1%).[26]

The DCCT demonstrated that improvement of glycemic control reduced nonproliferative and proliferative retinopathy (47% reduction), microalbuminuria (39% reduction), clinical nephropathy (54% reduction), and neuropathy (60% reduction). Improved glycemic control also slowed the progression of early diabetic complications. There was a nonsignificant trend in reduction of macrovascular events during the trial (most individuals were young and had a low risk of cardiovascular disease). The results of the DCCT predicted that individuals in the intensive diabetes management group would gain 7.7 additional years of vision, 5.8 additional years free from ESRD, and 5.6 years free from lower extremity amputations. If all complications of DM were combined, individuals in the intensive diabetes management group would experience 15.3 more years of life without significant microvascular or neurologic complications of DM, compared to individuals who received standard therapy. This translates into an additional 5.1 years of life expectancy for individuals in the intensive diabetes management group.[26]

The benefit of the improved glycemic control during the DCCT persisted even after the study concluded and glycemic control worsened. For example, individuals in the intensive diabetes management group for a mean of 6.5 years had a 42–57% reduction in cardiovascular events [nonfatal myocardial infarction (MI), stroke, or death from a cardiovascular event] at a mean follow-up of 17 years, even though their subsequent glycemic control was the same as those in the conventional diabetes management group.[26]

The benefits of an improvement in glycemic control occurred over the entire range of A1C values, suggesting that at any A1C level, an improvement in glycemic control is beneficial. The goal of therapy is to achieve an A1C level as close to normal as possible, without subjecting the patient to excessive risk of hypoglycemia.[26]

The United Kingdom Prospective Diabetes Study (UKPDS) demonstrated that each percentage point reduction in A1C was associated with a 35% reduction in microvascular complications. As in the DCCT, there was a continuous relationship between glycemic control and development of complications. Improved glycemic control did not conclusively reduce (nor worsen) cardiovascular mortality but was associated with improvement with lipoprotein risk profiles, such as reduced triglycerides and increased HDL.[27]

One of the major findings of the UKPDS was that strict blood pressure control significantly reduced both macro- and microvascular complications. In fact, the beneficial effects of blood pressure control were greater than the beneficial effects of glycemic control. Lowering blood pressure to moderate goals

(144/82 mmHg) reduced the risk of DM-related death, stroke, microvascular end points, retinopathy, and heart failure (risk reductions between 32 and 56%).[27]

Similar reductions in the risks of retinopathy and nephropathy were also seen in a small trial of lean Japanese individuals with type 2 DM randomized to either intensive glycemic control or standard therapy with insulin (Kumamoto study). These results demonstrate the effectiveness of improved glycemic control in individuals of different ethnicity and presumably, a different etiology of DM (that is phenotypically different from those in the DCCT and UKPDS).[27]

The findings of the DCCT, UKPDS, and Kumamoto study support the idea that chronic hyperglycemia plays a causative role in the pathogenesis of diabetic microvascular complications. These landmark studies prove the value of metabolic control and emphasize the importance of (1) intensive glycemic control in all forms of DM, and (2) early diagnosis and strict blood pressure control in type 2 DM.[27]

Renal complications of diabetes mellitus

Diabetic nephropathy, a relatively common microvascular complication of both type 1 and type 2 DM contributes maximally to the pool of patients with chronic renal failure. It is defined clinically as the presence of persistent proteinuria in a diabetic patient usually with retinopathy, elevated blood pressure and declining glomerular function, in the absence of UTI, other renal disease and/or heart failure.[25,28]

Figures from the U.S. renal data system, over the last three decades have shown a continual increase in the incidence of renal failure among patients with diabetes, predominantly with type 2 DM. This trend has been observed both in developed and developing countries. It is commoner to see more patients with type 2 DM with nephropathy, than those with type 1 DM (9:1) even though the incidence of nephropathy is higher in patients with type 2 DM. Recent data suggest that the incidence of end stage renal disease (ESRD) in patients with type 2 DM has increased dramatically and the reason for this change is due to the availability of better management options for hypertension and coronary artery disease in diabetic patients. As a result, more patients with type 2 DM live long enough for nephropathy and ESRD to develop. ESRD is patients with type 2 DM patients is therefore a disease of medical progress.[6,28,29,30]

Like other microvascular complications, the pathogenesis of diabetic nephropathy is related to chronic hyperglycemia. The mechanisms by which chronic hyperglycemia leads to ESRD, though incompletely defined, involve the effects of soluble factors (growth factors, angiotensin II, endothelin, AGEs), hemodynamic alterations in the renal microcirculation (glomerular hyperfiltration or hyperperfusion, increased glomerular capillary pressure), and structural changes in the glomerulus (increased extracellular matrix, basement membrane thickening, mesangial expansion, fibrosis). Some of these effects may be mediated through angiotensin II receptors. Smoking accelerates the decline in renal function. Because only 20–40% of patients with diabetes develop diabetic nephropathy, additional susceptibility factors remain unidentified. One known risk factor is a family history of diabetic nephropathy.[25,28,29]

The natural history of diabetic nephropathy is characterized by a fairly predictable sequence of events that was initially defined for individuals with type 1 DM but appears to be similar in type 2 DM. Glomerular hyperperfusion and renal hypertrophy occur in the first years after the onset of DM and are associated with an increase of the glomerular filtration rate (GFR). During the first 5 years of DM, thickening of the glomerular basement membrane, glomerular hypertrophy, and mesangial volume expansion occur as the GFR returns to normal. After five to ten years of type 1 DM, ~40% of individuals begin to excrete small amounts of albumin in the urine.[28,31]

Microalbuminuria is defined as 30–300 mg/d in a 24-h collection or 30 to 300 mg/gm creatinine in a spot collection (preferred method). Although the appearance of microalbuminuria in type 1 DM is an important risk factor for progression to overt proteinuria (>300 mg/d), only ~50% of individuals progress to macroalbuminuria over the next 10 years. In some individuals with type 1 diabetes and microalbuminuria of short duration, the microalbuminuria regresses. Once macroalbuminuria is present, there is a steady decline in GFR, and ~50% of individuals reach ESRD in 7–10 years. Once macroalbuminuria develops, blood pressure rises slightly and the pathologic changes are likely to be irreversible. Some individuals with type 1 or type 2 DM have a decline in GFR in the absence of micro- or macroalbuminuria and this is the basis for assessing the GFR on an annual basis using serum creatinine.[32-35]

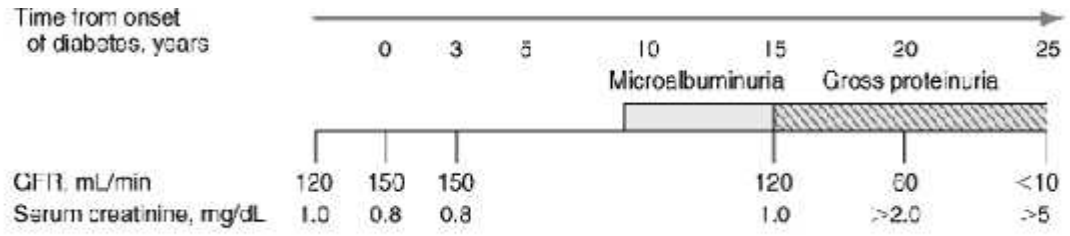


Figure 2. Time course of development of diabetic nephropathy

The nephropathy that develops in type 2 DM differs from that of type 1 DM in the following respects: (1) Microalbuminuria or macroalbuminuria may be present when type 2 DM is diagnosed, reflecting its long asymptomatic period; (2) Hypertension more commonly accompanies microalbuminuria or macroalbuminuria in type 2 DM and (3) Microalbuminuria may be less predictive of diabetic nephropathy and progression to macroalbuminuria in type 2 DM. Finally, it should be noted that albuminuria in type 2 DM may be secondary to factors unrelated to DM, such as hypertension, congestive heart failure (CHF), prostate disease, or infection. Diabetic nephropathy and ESRD secondary to DM develop more commonly in African Americans, Native Americans, and Hispanic individuals than in Caucasians with type 2 DM. Microalbuminuria is associated with other Microvascular complications as well as with cardiovascular disease suggesting some common pathophysiological mechanisms.[32]

In the past three decades, urinary albumin excretion has assumed a central role in the diagnosis and management of kidney disease among people with diabetes, both type 1 and type 2. Microalbuminuria was initially found to predict subsequent over albuminuria (more than 300mg/24h), which in turn predicted loss of GFR. From the strength of these relationships it has frequently been

assumed that microalbuminuria and overt albuminuria are requisite first and second steps along a single pathway that leads to loss of GFR and ESRD. Persistent microalbuminuria was strong risk factor for subsequent loss of GFR, reemphasizing earlier work that established the importance of sustained increases in urine albumin excretion in the pathogenesis and diagnosis of diabetic kidney disease.[30,36]

However, patients who lost GFR at a high rate did not have overt albuminuria, by study design, and some had 'normal' urinary excretion of albumin. This study contributes to a growing literature that suggest that overt albuminuria does not always precede a significant loss of GFR in the setting of diabetes and that measuring albuminuria alone does not fully capture the scope of early diabetic kidney disease. Instead, albuminuria and GFR loss may represent complementary, if overlapping, manifestations of kidney damage.[37,38]

The National Kidney Disease Education Program and the National Kidney Foundation now recommended the use of estimating equations to improve the diagnostic accuracy of serum creatinine. These recommendations constitute a large step forward, with GFR most often estimated using the abbreviated Modification of Diet in Renal Disease (MDRD) equation.[39]

A doubling of serum creatinine level indicates a halving of GFR; a threefold increase suggests a 75% loss of kidney function. It is now clear that stage 3 or higher chronic kidney disease occurs in the absence of urine albumin excretion in a substantial proportion of adults with diabetes. Screening this

population for increased urine albumin excretin alone therefore, will miss a considerable number of chronic kidney disease cases.[35,37,40]

GFR estimation is the only renal parameter which can singly provide a picture of the actual renal status of Type 2 DM patients at any duration irrespective of the status of albuminuria, azotemia or renal size and morphology as their variability or progression is non-linear.[36,40]

End stage renal disease as a percentage is still very low, the cause of which is probably increased cardiovascular mortality and not a better management strategy as the prevalence of albuminuria (micro and macro) is still very high in our population. We can expect a higher percentage of ESRD in the coming decade if cardiovascular mortality improves.[13,36]

In overt diabetic nephropathy an overall decrease of glomerular filtration rate is present at the same time with proportional enhancement of albumin filtering. How ever, when progression to the renal failure stage is achieved further huge drop of GFR, but only negligible urine albumin excretion increase is evidenced due to total occlusion of a significant proportion of glomeruli.[25]

Although albuminuria is claimed to be a marker of nephropathy progression, at this stage it fails to reflect appropriately glomerular function and could not be used as its valuable parameter.[35,41]

However, previous studies concluded that higher levels of albumin creatinine ratio even within the normal range was associated with faster decline in eGFR in diabetic patients.[39]

Related to albuminuria, the results of previous study favour that the glomerular filtration rate estimation to define kidney disease stage in diabete mellitus patients.[42]

Role of lipids in diabetic nephropathy

In comparison to normoalbuminuric diabetic patients, those with microalbuminuria and overt proteinuria have been reported to have significantly higher plasma concentrations of very low density lipoprotein (VLDL), low density lipoprotein (LDL) and triglyceride but lower levels of high density lipoprotein cholesterol (HDL). No major compositional changes in lipoproteins have been observed between patients with microalbuminuria and albuminuria. Elevated plasma concentrations of apolipoprotein B, apoC-III and apoprotein(a), and increased mass concentrations of the highly atherogenic intermediate-density lipoprotein fraction (IDL) have also been reported in patients with both microalbuminuria and albuminuria in comparison to normoalbuminuric diabetic subjects.[43]

Furthermore, the diameter of LDL particles, which is strongly regulated by plasma triglyceride level, has been reported to be smaller in patients with diabetic nephropathy, including those with microalbuminuria, as compared to diabetic patients without nephropathy. The postprandial lipemia has been shown to be significantly greater in diabetic patients with nephropathy when compared with normoalbuminuric patients, suggesting that the reduction of LDL particle size in diabetic nephropathy is closely related to early modification of triglyceride-rich lipoproteins metabolism.[43]

An increase in hepatic lipase (HL) activity and a reduced postheparin plasma lipoprotein lipase (LPL)/HL ratio have been reported in micro and macroalbuminuric patients as compared with normoalbuminuric diabetic subjects. All these multiple lipoprotein abnormalities described in diabetic patients with nephropathy become more accentuated with decreasing renal function and increasing urinary albumin excretion.[43]

Potential influence of lipids in the pathogenesis of diabetic nephropathy

Experimental studies

Previous experimental studies[44-47] have reported a link between hyperlipidemia and the formation of glomerulosclerosis. Dietary induced hypercholesterolemia in rats, guinea pigs and rabbits was associated with a premature development of focal glomerulosclerosis.

Experiments in the obese Zucker rat which represents a model of dyslipidemia with peripheral insulin resistance, showed that hyperlipidemia may induce glomerular injury, especially focal glomerular sclerosis, independently of glomerular hemodynamics.[43]

Furthermore lipid-lowering treatment has been shown to attenuate renal lesions in Zucker rats and in the subtotal nephrectomy model, emphasizing a possible pivotal role for lipids in the pathogenesis of progressive renal disease.[43]

The influence of hyperlipidemia on renal function may be even more important in the presence of other risk factors like arterial hypertension, renal

ablation or diabetes mellitus.[43,45] The mechanisms by which lipids cause or exacerbate glomerular injury remain incompletely understood.

Mechanisms of lipid-induced renal injury

In the glomerulus

In human glomeruli, both mesangial and epithelial cells take up lipoproteins via specific receptors. Mesangial cells also express scavenger receptors which are involved in the preferential uptake of modified, glycosylated and oxidized LDL, as observed in diabetes. Accumulation of modified LDL in the mesangium or in mesangial matrix has been reported to favor their uptake by infiltrated glomerular monocytes, leading in turn to the subsequent activation of these cells into macrophages. This preferential phagocytosis of modified LDL by monocytes has been also reported to play a pivotal role in the formation of mesangial foam cells. Accumulated mesangial modified lipoproteins may influence the pathogenesis of glomerulosclerosis by different mechanisms: Exposure to oxidized lipoproteins has been reported to stimulate mesangial cell secretion of various chemotactic factors and adhesion molecules (M-CSF, ICAM-1, VCAM-1), enhancing the renal recruitment of macrophages. These factors result in monocyte infiltration which has been reported to play a key role in the pathogenesis of glomerulosclerosis and tubular fibrosis, in particular in diabetic nephropathy. These intramesangial recruited macrophages may in turn further oxidize LDL, creating a vicious self perpetuating cycle resulting in progressive renal injury. Renal activated macrophages have been shown to stimulate the release of reactive oxygen species and the expression of prosclerotic and

proliferative cytokines such as transforming growth factor 1 (TGF-1) and platelet-derived growth factor-AB (PDGF-AB). These cytokines stimulate the production of extra-cellular matrix proteins, promoting mesangial expansion as has been described in diabetic nephropathy. In vitro studies have demonstrated that LDL and oxidized LDL stimulate TGF-_1 gene expression in both human glomerular mesangial and epithelial cells.

Therefore, TGF-1 appears to be an important mediator of lipid-induced mesangial matrix expansion as well as playing a key role in the pathogenesis of diabetic nephropathy. Finally, the uptake of modified LDL by macrophages has been reported to stimulate eicosanoid synthesis including thromboxanes and leukotrienes, leading to potentially deleterious alterations in intra-glomerular hemodynamics. In this regard, dietary cholesterol supplementation in animals has been shown to result in an increase in efferent arteriole resistance and a subsequent elevation in intra-glomerular pressure. This effect may exacerbate glomerular injury in diabetic nephropathy. However, one must be cautious in extrapolating the findings of these studies to human diabetic nephropathy since most of the *in vivo* studies reported above were conducted in non diabetic models of renal disease, with a focus on focal glomerulosclerosis.

Indeed, it has been reported that dietary cholesterol supplementation in Sprague Dawley rats with streptozotocin-induced diabetes did not influence urinary albumin excretion and any glomerular ultrastructural parameter. Nevertheless, the data from these experimental studies in different models may be relevant, at least in part, for the pathogenesis of lipid induced renal injury in diabetic nephropathy.

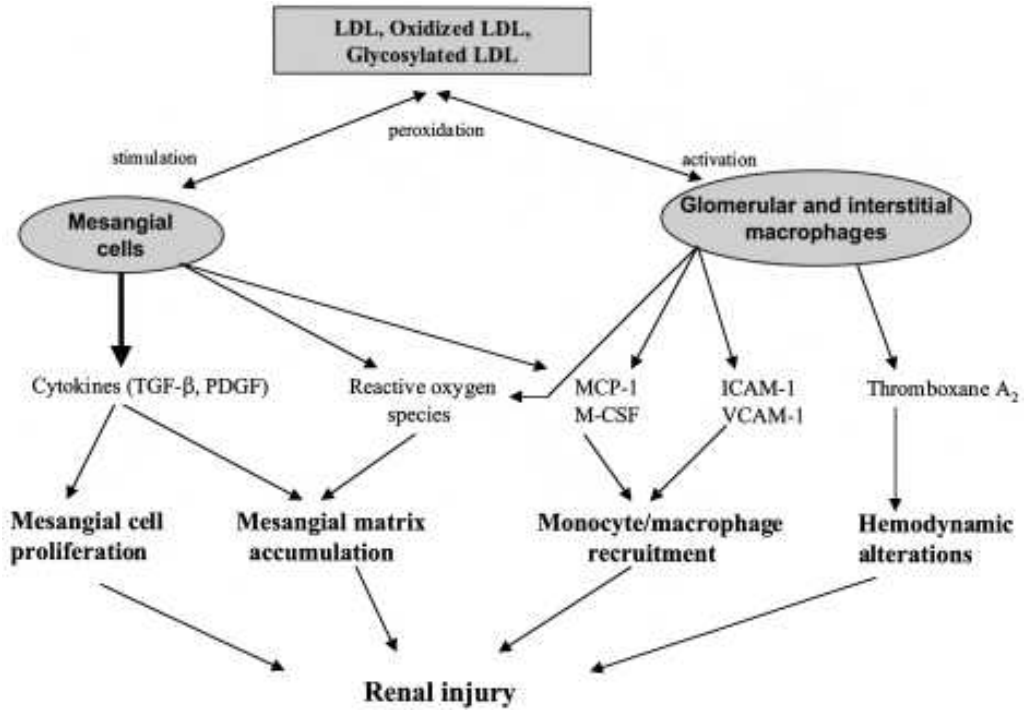


Figure 3. Schematic representation of possible pathophysiological mechanisms mediating lipid-induced renal injury.

In the tubulointerstitium

Tubulointerstitial injury has been clearly demonstrated over the last decade to play a pivotal role in the pathogenesis of diabetic nephropathy and to be an important predictor of renal dysfunction.[48] Animal studies have demonstrated a damaging effect of hyperlipidemia on the tubulointerstitium.[49] Although it had been reported in hyperlipidemic Zucker rats that tubulointerstitial injury closely paralleled the development of glomerulosclerosis,- other authors[49,50] have described that lipid-induced tubulointerstitial lesions may precede glomerular changes or occur independently of the glomerular lesions. In these experimental studies,[49,51] hyperlipidemia-induced chronic tubulointerstitial damage was associated with significant interstitial macrophage infiltration and a

parallel increase in TGF-1 gene expression in interstitial cells, suggesting a cytokine-mediated role for lipids in the development or aggravation of tubulointerstitial lesions.

Furthermore, in proteinuric conditions such as overt diabetic nephropathy, it has been proposed from experimental *in vivo* studies[52] that the tubular uptake and metabolism of the lipid component of filtered lipoproteins lead to local expression of chemokines and cytokines and promote interstitial inflammation.

Potential interactions between angiotensin II and the lipid nephrotoxicity in diabetic nephropathy

There is evidence suggesting activation of the renin-angiotensin system (RAS) in the kidney in diabetes. In particular, the major effector peptide of this pathway, angiotensin II, is viewed to play a pivotal role in the pathogenesis of diabetic nephropathy. There may be an interplay of lipoproteins and angiotensin II-induced renal injury pathways in diabetic nephropathy. Some of the pathophysiological mechanisms of lipid induced nephrotoxicity are also observed with angiotensin II-induced renal disease. Firstly, angiotensin II has been showed to increase oxidant stress, and this would favor the formation of oxidized LDL which plays a key role in the formation of renal lesions.[43]

Secondly, angiotensin II increases microvascular glomerular permeability and may therefore enhance glomerular macromolecules traffic and lead to both mesangium lipid accumulation and tubular lipoprotein overload. Lastly, it has been demonstrated that angiotensin II stimulates gene expression of many cytokines and chemokines, potentially enhancing lipid-induced macrophage

infiltration, extracellular matrix accumulation and promoting the subsequent formation of renal injury.

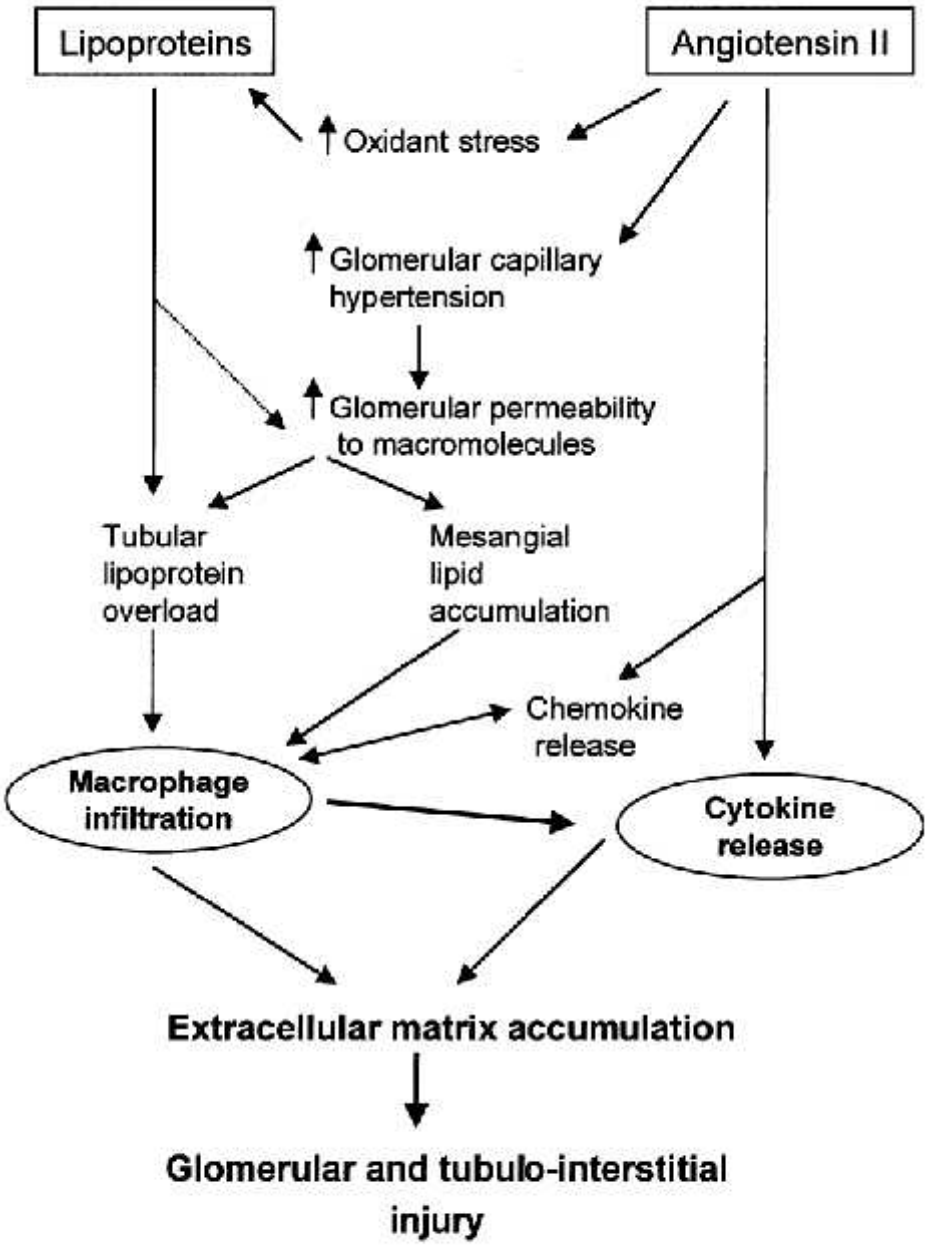


Figure 4. Potential interactions between lipoproteins and angiotensin II in the pathogenesis of diabetic nephropathy[43]

Furthermore, experimental studies[53] have showed that ACE inhibitors may reduce lipid induced glomerular lesions in obese diabetic rats. These experimental findings are consistent with clinical data which have suggested that in diabetic nephropathy, the specific deleterious influence of serum cholesterol was markedly attenuated by ACE inhibitor treatment.

A study[54] found that in type 1 diabetic patients, the ACE inhibitor treatment, in contrast to the beta blocker metoprolol, was associated with a marked reduction in the decline of renal function over 2.5 years. This renoprotective effect was also observed in the subgroup of patients with baseline hypercholesterolemia. ACE inhibitor treatment and serum cholesterol levels were two mutually independent risk factors for the subsequent decline in renal function. These data suggest additive effects of these two parameters on loss of renal function.

Furthermore, a reduction in cholesterol secondary to the antiproteinuric effect of ACE inhibitor could further enhance the beneficial renal effects of these agents.[56]

Role of lipids in the development of diabetic nephropathy

Only a few studies have prospectively assessed the potential influence of initial serum lipid levels on the subsequent development of incipient or overt diabetic nephropathy. The close interrelationships between serum lipids values, blood glucose status and proteinuria make more difficult to determine the influence of lipids per se in the progression of diabetic nephropathy, particularly in uncontrolled observational studies.[43]

In type 1 diabetes

A study[47] reported in 53 normoalbuminuric type 1 diabetic patients that the increase in albuminuria after 10 years follow-up was significantly and positively related to the baseline serum total cholesterol, LDL cholesterol and apolipoprotein B levels. In that study, no significant association was found with triglycerides, HDL cholesterol, apo A-I and Lp(a) levels. Among the variables entered in a multivariate statistical analysis, only initial serum apo B level was an independent predictor of the progression of very low-level albuminuria. However, only 5 patients progressed to microalbuminuria, potentially limiting the relevance of these findings.

In type 2 diabetes

In a prospective observational study[58] with a mean 5.8 years follow-up, from the Steno Diabetes Center have evaluated the main risk factors for the development of persistent micro and macroalbuminuria in 191 normoalbuminuric type 2 diabetic patients. They found in a multivariate analysis that baseline concentration of serum cholesterol, but not HDL cholesterol, was an independent risk factor for the development of incipient or overt diabetic nephropathy. The potential influence of serum triglycerides was not evaluated in that study. That study[59] agrees with findings observed in Pima Indians, in whom serum cholesterol level was found to be related to the development of increased urinary albumin excretion.

More recently another study[60] provided further evidence for an independent role of plasma cholesterol in the subsequent loss of renal function

and increase in urinary albumin excretion in diabetic subjects without nephropathy. In an uncontrolled prospective study,[60] 574 patients, initially normotensive with both normal renal function and urinary albumin excretion rate were followed-up for a mean period of 7.8 years. In a multiple logistic regression analysis, baseline serum total cholesterol level, mean blood pressure and glycosylated hemoglobin were the main independent determinants of the subsequent decline in renal function (assessed as the reciprocal creatinine value). The risk to develop microalbuminuria was also independently predicted by the baseline values of total cholesterol, mean blood pressure, glycosylated hemoglobin, HDL cholesterol and the body mass index.

Role of lipids in the progression of diabetic nephropathy

In both type I and type II diabetes, only a few prospective and case-control studies have attempted to establish a correlation between hyperlipidemia and the decline of renal function in diabetic nephropathy. However most of these studies were post-hoc secondary analyses of previous controlled trial assessing the renal effects of angiotensin converting enzyme (ACE) inhibitors in diabetic nephropathy.[43]

Although lipid-lowering treatments have been shown to be effective in diabetic subjects and to reduce cardiovascular morbidity and mortality in the presence of moderate hyperlipidemia,[61] their potential renoprotective properties in diabetic nephropathy as well as in nondiabetic chronic renal diseases remain still controversial.

In type I diabetes

A study[54] prospectively studied over 2.5 years the relationship between serum lipid values and the subsequent decline in glomerular filtration rate (GFR) in 30 type I diabetic patients with already advanced renal disease and proteinuria at the time of commencement of the study. All patients were on antihypertensive treatment with either enalapril or metoprolol. These investigators demonstrated that the decline in GFR was negatively correlated to initial values of plasma total cholesterol, triglycerides and apolipoprotein B and was positively correlated to Apo AI. There was no correlation between Lp(a) level and the decline in renal function. In a stepwise regression analysis taken into account multiple covariates, including glycosylated hemoglobin, arterial blood pressure and albuminuria, the strongest factors linked to decline in GFR were serum cholesterol and the type of antihypertensive treatment.

Another study[62] found a significant positive correlation in univariate analysis between serum cholesterol level and the rate of decline in GFR in a 10 year prospective study of ACE inhibitors in diabetic nephropathy. These patients were all hypertensive with persistent albuminuria without evidence of chronic renal failure. However this association with serum cholesterol levels did not remain significant after a stepwise multiple regression analysis which included other variables such as mean arterial blood pressure, albuminuria and glycemic control.

A study[63] in a post-hoc analysis of the data collected during the prospective Diabetic Retinopathy Study, examined the determinants of

progression to chronic renal failure (assessed by serum creatinine) in 439 patients with diabetic nephropathy. All these patients had severe diabetic retinopathy and either intermittent or persistent proteinuria and were followed-up for more than 3 years. Only one third of the patients experienced a rapid loss of renal function. Among the different baseline variables analyzed, only serum cholesterol level and diastolic blood pressure at entry were significantly associated with a rapid loss of renal function. These correlations remained significant in a multiple logistic regression analysis.

In type II diabetes

A study[55] followed prospectively for five years 94 normotensive patients with microalbuminuria and normal renal function. These patients were randomized to receive either enalapril or placebo. These authors reported a significant correlation between baseline and mean study values of serum total cholesterol and the subsequent evolution of renal function (expressed as the ratio of initial to final reciprocal serum creatinine values). This association persisted after stratification for blood pressure and was observed in both the enalapril and placebo treated groups. In the placebo as well as in enalapril treated patients, initial and mean plasma total cholesterol and mean blood pressure were also significant predictors of the subsequent increase in albuminuria. No correlation was found in that study between serum HDL cholesterol or triglyceride levels and either the renal outcome or the increase in albuminuria. However, other investigators have reported in type 2 diabetes, a significant independent influence of the serum triglyceride level on progression of microalbuminuria and progressive loss of renal function in diabetic nephropathy. Furthermore, in these

studies, serum cholesterol did not emerge as a significant predictor of renal outcome. Lastly, it should be noted that a number of other observational studies have failed to demonstrate any independent effect of any serum lipid parameter on either evolution of albuminuria or the decline in renal function.

A cross-sectional analysis[64] of 5808 elderly participants in the Cardiovascular Heart Study (CHS) also revealed higher triglycerides levels in those with renal insufficiency (defined as sCr ≥1.5 mg/dl in men and ≥1.3 mg/dl in women) compared to those without renal insufficiency; participants with diabetes comprised only 12–14% of this cohort.

A recent prospective analysis[65] of 4,517 members of Physicians' Health Study demonstred that those with an HDL < 40 mg/dl at baseline had a two-fold higher risk of renal insufficiency (defined a serum creatinine ≥ 1.5 mg/dl) at 14 years of follow-up.

Previously published studies[66,67] on the association between LDL and presence of kidney disease have been mixed, with a couple of investigations reporting a positive association, and others reporting no association.[7,64,67] The finding that LDL was inversely associated with GFR < 60 ml/min/1.73 m^2 was opposite to our expectations especially because in our cohort, the highest quartile of LDL was associated with a −2.8 ml/min/1.73m^2 difference in estimated GFR when compared to the lowest quartile. On closer scrutiny, these OR estimates were based on only a few patients who fell within the highest quartile of LDL and had estimated GFR < 60 ml/min/1.73 m^2. LDL was not statistically associated

with estimated GFR in any of our other analyses, and therefore, we felt this represented a chance finding.[10]

A study[69] on 516 women with type 2 diabetes in the Nurses' Health Study with data on lipid and inflammatory biomarkers from plasma collected in 1989 and plasma creatinine in samples collected in 1989 and 2000. An estimated GFR decline of >or=25% over 11 years was the outcome of interest. Comparing the highest with the lowest quartile, soluble tumour necrosis factor receptor 2 (sTNFR-2) was independently associated with an eGFR decline of >or=25% (multivariate OR 5.81; 95% CI 2.90-11.65); this association was stronger in obese women (OR 16.76; 95% CI 4.69-59.90 for BMI >or=30 kg/m(2); OR 2.78, 95% CI 1.12-6.89 for BMI <30 kg/m(2); p for interaction = 0.02). No lipids (total cholesterol, LDL-cholesterol, HDL-cholesterol, non-HDL-cholesterol, triacylglycerols, lipoprotein(a), or apolipoprotein B) were significantly associated with eGFR decline after multivariable adjustment. Study concluded that, elevated sTNFR-2 levels may be an important and potentially modifiable risk factor for eGFR decline in type 2 diabetes, especially in those with a BMI of >or=30 kg/m(2).

Diabetic nephropathy screening – As per ADA Clinical practice Recommendations 2006 – perform an annual test for the presence of microalbuminuria in type 1 diabetic patients with diabetes duration of more than five years and in all type 2 diabetic patients, starting at diagnosis and during pregnancy.[70]

C-reactive protein

C-reactive protein is a protein found in the blood, the levels of which rise in response to inflammation (an acute-phase protein). Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via the C1Q complex.[71] CRP is synthesized by the liver[72] in response to factors released by adipocytes.[73] It is a member of the pentraxin family of proteins.

History

CRP was originally discovered by Tillett and Francis in 1930 as a substance in the serum of patients with acute inflammation that reacted with the *C* polysaccharide of pneumococcus.[74] Initially it was thought that CRP might be a pathogenic secretion as it was elevated in people with a variety of illnesses including cancer, however discovery of hepatic synthesis demonstrated that it is a native protein.

Genetics

The *CRP* gene is located on the first chromosome (1q21-q23). CRP is a 224-residue protein with a monomer molar mass of 25106 Da. The protein is an annular pentameric disc in shape and a member of the small pentraxins family.

Function

CRP is a member of the class of acute-phase reactants, as its levels rise dramatically during inflammatory processes occurring in the body. This

increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages as well as adipocytes. CRP binds to phosphocholine on microbes. It is thought to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages, which express a receptor for CRP. It is also believed to play another important role in innate immunity, as an early defense system against infections.

CRP rises up to 50,000-fold in acute inflammation, such as infection. It rises above normal limits within 6 hours, and peaks at 48 hours. Its half-life is constant, and therefore its level is mainly determined by the rate of production and hence the severity of the precipitating cause.

Diagnostic use

CRP is used mainly as a marker of inflammation. Apart from liver failure, there are few known factors that interfere with CRP production.[72] Measuring and charting CRP values can prove useful in determining disease progress or the effectiveness of treatments. Various analytical methods are available for CRP determination, such as ELISA, immunoturbidimetry, rapid immunodiffusion, and visual agglutination.

A high-sensitivity CRP (hs-CRP) test measures low levels of CRP using laser nephelometry. Normal concentration in healthy human serum is usually below 3 mg/L. Higher levels are found in late pregnant women, inflammation, viral infections, bacterial infections and burns.[75]

Baseline C-reactive protein (CRP) levels add to the predictive value of lipid parameters in determining the risk of first myocardial infarction in apparently healthy men and women without a history of coronary heart disease. Baseline CRP levels also were found to be predictive of symptomatic peripheral vascular disease in a cohort of healthy men. CRP reflects systemic inflammation, and these results support the hypothesis that chronic inflammation may play a role in the pathogenesis and progression of atherosclerosis.

INTERLEUKIN-18 (IL-18)

Interleukin-18 a recently described member of the IL-1 cytokine superfamily, is now recognized as an important regulator of innate and acquired immune responses. IL-18 is expressed at sites of chronic inflammation, in autoimmune diseases, in a variety of cancers, and in the context of numerous infectious diseases.[76]

The cytokine is produced constitutively in many different cell types, including macrophages, endothelial cells, vascular smooth muscle cells, dendritic cells and Kupffer cells. IL-18 is also produced in adipocytes, but non-adipocyte cells have been identified as the main source of IL-18. In adipose tissue, IL-18 is produced as a 24-kD inactive precursor lacking a signal peptide (pro-IL-18). Pro-IL-18 is cleaved after Asp35 by the endoprotease IL-1β-converting enzyme (ICE; caspase-1) to generate a biologically active, mature 18-kD moiety.[76]

Once secreted, IL-18 is bound and inactivated by IL-18 binding protein, which is enhanced as a negative feedback mechanism in response to increased IL-18 production, ensuring protection from tissue damage due to uncontrolled

proinflammatory activity.[76] IL-18 binds to its receptor, consisting of an α chain which is responsible for extracellular binding of IL-18, and a β chain which is responsible for intracellular signal transduction.[76] Although both free and protein-bound IL-18 may bind to the α chain, only the free fraction is able to activate the β chain.[76]

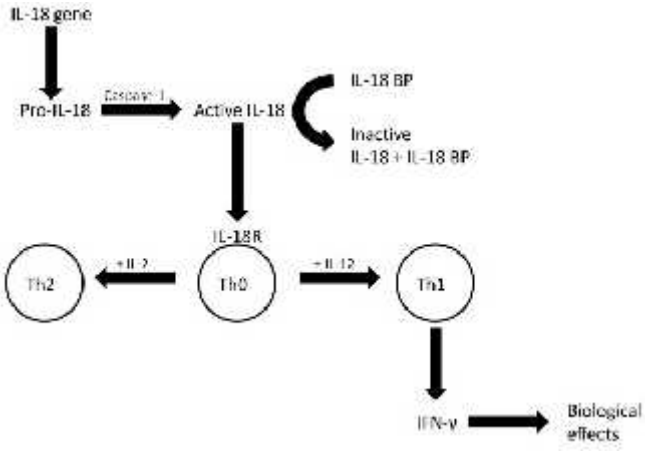


Figure 5. Regulation and biological effects of interleukin-18

Biological effects of IL 18

Although originally identified as a factor capable of inducing IFN-γ production by murine splenocytes, the effector role of IL-18 is rapidly expanding.[77]

IL-18 is a potent proinflammatory cytokine which enhances T cell and natural killer cell maturation, as well as the production of cytokines, chemokines and cell adhesion molecules. IL-18 can promote Th1 or Th2 lineage maturation dependent on underlying genetic influences and the ambient cytokine milieu. IL-18 promotes neutrophil activation, reactive oxygen intermediate synthesis, cytokine release, and degranulation.

Recent studies[78] suggest that IL-18 up-regulates intracellular adhesion molecule-1 (ICAM-1) and VCAM-1 expression on endothelial cells and synovial fibroblasts. IL-18 is particularly effective during the clearance of intracellular bacteria, fungi, and protozoa, requiring the induction of host-derived IFN-γ, which in turn evokes effector pathways involving molecules such as nitric oxide (NO). IL-18 also plays a part in the clearance of viruses, partly through the induction of cytotoxic T cells. IL–18 may act synergistically with IL-12 to stimulate a Th1 response with production of IFN-γ, a central feature in the atherosclerotic lesion.[76]

IL 18 and Atherosclerosis

Several lines of evidence support a pro-atherogenic role for IL-18. Expression of IL-18 and the IL-18 receptor subunits are increased in atherosclerotic arteries compared with normal arterial segments. Stimulation of cell types found in atheromata with IL-18 induces proinflammatory cytokines such as interferon (IFN)-γ and IL-6, adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), and enzymes capable of degrading extracellular matrix metalloproteinase (MMPs). In addition, studies in mice demonstrate that lack of IL-18 or inhibition of IL-18 signaling decreases atherosclerotic plaque formation, whereas administration of exogenous IL-18 promotes atherogenesis and yields more fatty than fibrous lesions.[79]

Literature on role of IL-18 and hs-CRP in diabetic nephropathy

A cross-sectional study[10] of 732 men with type II DM enrolled in the Health Professionals' Follow-Up Study at Massachusetts, USA used plasma

creatinine to estimate GFR by the simplified Modification of Diet in Renal Disease (MDRD) equation. An inverse association was observed for HDL (OR 0.48; 95% CI 0.24-0.98). Study found no association between C-reactive protein and GFR. The results were similar when creatinine clearance by Cockcroft-Gault was used to estimate kidney function. Study concluded that several potentially modifiable lipid and inflammatory biomarkers are elevated in the setting of moderately decreased GFR in men with type II DM and may be the link between renal insufficiency and increased risk for cardiovascular events in this population.

Recent investigations have reported that higher baseline levels of sTNFR-2, IL-6, CRP,[80] E-selectin, and ICAM[81] are independent predictors of incident type 2 diabetes in prospective nested case-control analyses of the Nurses' Health Study. The accumulating evidence that a chronic inflammation is a harbinger of adult-onset diabetes raises provocative questions of which, if any, of these same mechanisms lead to renal dysfunction in diabetes and whether any of these mechanisms also underlie non-diabetic nephropathy.

Some published investigations[7-9] have found higher CRP levels in those with chronic renal failure or on dialysis compared to healthy controls, it should be noted that diabetic subjects were excluded or comprised a minority (11 to 13%) of the study subjects.

A study[69] on 516 women with type 2 diabetes in the Nurses' Health Study with data on lipid and inflammatory biomarkers from plasma collected in 1989 and plasma creatinine in samples collected in 1989 and 2000. An estimated GFR decline of >or=25% over 11 years was the outcome of interest. Comparing the

highest with the lowest quartile, soluble tumour necrosis factor receptor 2 (sTNFR-2) was independently associated with an eGFR decline of >or=25% (multivariate OR 5.81; 95% CI 2.90-11.65); this association was stronger in obese women (OR 16.76; 95% CI 4.69-59.90 for BMI >or=30 kg/m(2); OR 2.78, 95% CI 1.12-6.89 for BMI <30 kg/m(2); p for interaction = 0.02). Markers of inflammation (C-reactive protein, fibrinogen, E-selectin, intracellular cell adhesion molecule 1, leptin or adiponectin) were significantly associated with eGFR decline after multivariable adjustment. Study concluded that, elevated sTNFR-2 levels may be an important and potentially modifiable risk factor for eGFR decline in type 2 diabetes, especially in those with a BMI of >or=30 kg/m(2).

Chapter 4

Methodology

METHODOLOGY

The present study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on patients with type 2 diabetes mellitus.

Study design

The study design was one year cross sectional study.

Study period and duration

The present one year study was conducted during the period of January 2011 to December 2011.

Place

This study was conducted at Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum a teaching hospital attached to Jawaharlal Nehru Medical College, Belgaum.

Source of Data

Patients with diabetes mellitus admitted in the wards of Medicine Department or attending the Medicine OPD were studied.

Sample size

A total of 100 patients with type diabetes mellitus were selected for the study.

Sampling procedure

The sample size was calculated based on the formula as mentioned below.

$n = 4 X p X q / d^2$

Where p = Prevalence (Prevalence of the disease which was taken as 50% as no records were available regarding the study).

q = 100 – p

d = Absolute error taken as 10%

$n = 4 X 50 X 50 / 10^2$

n = 100

Sampling method

Simple random sampling was employed where every third patient who fulfilled the selection criteria was included in the study.

Selection criteria

Inclusion Criteria

- Patients with type 2 diabetes mellitus.

Exclusion Criteria

- Type 1 diabetes mellitus.
- Participants who are on dialysis or had a kidney transplant.
- Patients taking statins and ACE inhibitors.
- Acute Febrile illness.

- Urinary tract infection.
- Congestive heart failure.
- Hypertension.
- Ischaemic heart disease.
- Acute coronary syndromes.

Ethical clearance

The study was approved by the Ethical and Research Committee, Jawaharlal Nehru Medical College, Belgaum.

Informed Consent

All the patients fulfilling selection criteria were explained about the purpose of study and a written informed consent was obtained before enrollment (Annexure I).

Method of collection of data

Demographic data such as age, sex, occupation, history regarding hypertension, diabetes mellitus and complications, cerebrovscular events viz, angina pain, myocardial infarction, ischaemic disease were recorded. A thorough physical examination such as anthropometry, vitals and systemic examination was conducted. These findings were recorded on a predesigned and pretested proforma (Annexure II).

Procedure

A thorough clinical examination was conducted and the findings were also recorded.

Body mass index was calculated based on formula;

$$\text{Body Mass Index} = \frac{\text{Weight (Kg)}}{\text{Height}^2 \text{ (m)}}$$

Body mass index in the range of less than 18.5 kg/m^2 were considered as underweight, 18.5 to 24.9 kg/m^2 were considered as normal, 25.0 to 29.9 kg/m^2 were considered as overweight and more than 30 kg/m^2 were considered as obese.[73-75]

The waist circumference was measured using a standard measuring tape in Cms. Waist circumference of ≥ 90 cms in males and ≥ 80 cms in females was considered as abnormal.

Investigations such as fasting blood sample for estimation of IL-18, hs-CRP, blood glucose, HbA1C, lipid profile (total cholesterol, triglycerides, HDL, LDL) and were done. Others tests like electrocardiogram were done wherever indicated.

Specific investigations

Urine Micro-Albumin Excretion(UAE) Test (Microalbumin to creatinine Ratio)

Urine Micro-Albumin was done by immunoturbidometry and urine creatinine was done by Jaeffe's method. The interpretation was done as below.

Traces	0.00 to 0.30
1+	0.30 to 1.00
2+	1.00 to 5.00
3+	> 5.00

1. Serum Creatinine (to calculate eGFR by MDRD Formula)

    a. Serum creatinine (by Jaeffe's method)

Based on the MDRD formula eGFR was calculated and patients were staged as below.

Stage	eGFR	Extent of kidney damage
I	90+	Normal or minimal kidney damage with normal GFR
II	60-89	Mild decrease in GFR
III	30-59	Moderate decrease in GFR
IV	15-29	Severe decrease in GFR
V	<15	Kidney failure

Estimation of IL-18 was done using standard recombinant IL-18 enzyme linked immuno sorbent assay (ELISA) kit. Interleukin-18 levels above 216 pg/mL were considered as abnormal.[76]

Statistical analysis

The data obtained was coded and entered into Microsoft Excel Worksheet. The categorical data was expressed as rates, ratios and proportions and comparison was done using chi-square test. The continuous data was expressed as mean ± standard deviation (SD) and the comparison was done using unpaired 't' test. A probability value ('p' value) of less than or equal to 0.05 was considered as statistically significant.

Chapter 5

Results

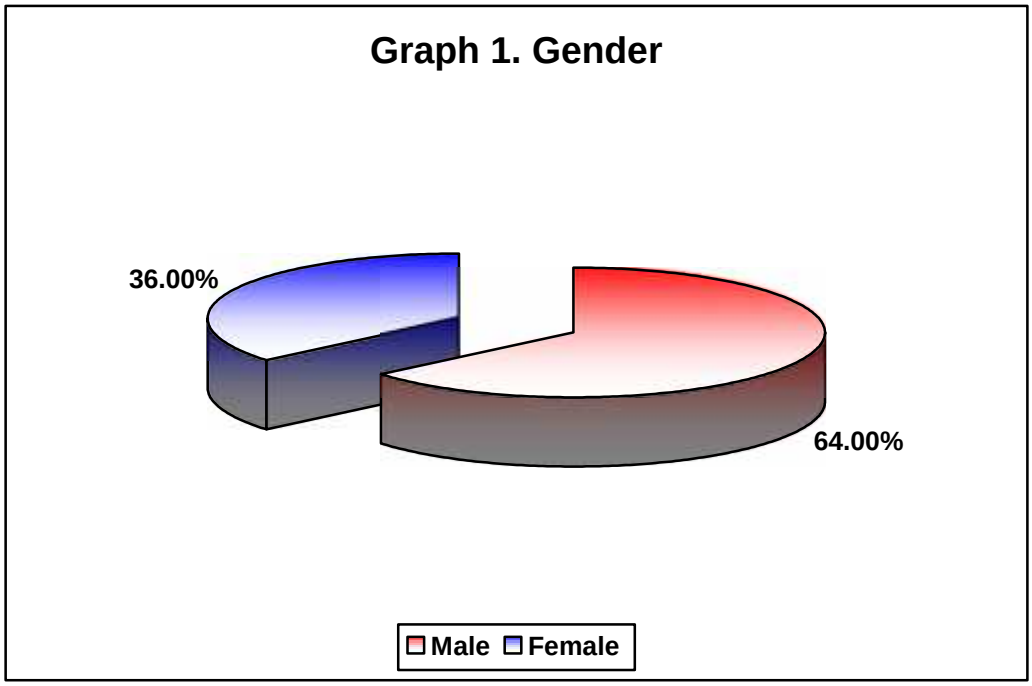
RESULTS

The present one year cross sectional study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. A total of 100 patients with type diabetes mellitus were selected for the study. Based on the simple random sampling, every third patient who fulfilled the selection criteria was included in the study.

The data obtained was coded and entered into Microsoft Excel Worksheet. Data was analyzed and results were tabulated as below.

Table 1. Gender

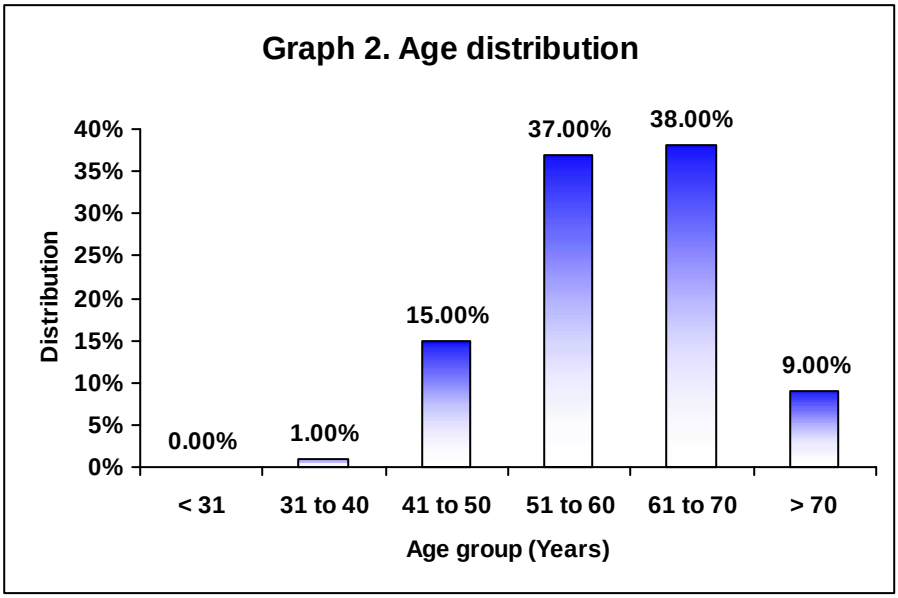
Gender	Distribution (n=100)	
	Number	**Percentage**
Male	64	64.00
Female	36	36.00
Total	**100**	**100.00**



In the present study, males accounted for 64% (n=64), whereas females accounted for 36% (n=36).

Table 2. Age distribution

Age group (Years)	Distribution (n=100)	
	Number	**Percentage**
< 31	0	0.00
31 to 40	1	1.00
41 to 50	15	15.00
51 to 60	37	37.00
61 to 70	38	38.00
> 70	9	9.00
Total	**100**	**100.00**



The above table shows the distribution of subjects according to age. Majority of the subjects were between 61 and 70 years accounting for 38% (n=38), with 15 and 37 subjects between 41-50 and 51-60 respectively. Only a small number were below 40 or above 70 years (n=1, n=1 respectively). The mean age for the study population was 60.3 ± 9.43 years.

Table 3. Duration of Type 2 diabetes

Duration (Years)	Distribution (n=100)	
	Number	**Percentage**
Upto 5	27	27.00
6 to 10	36	36.00
11 to 15	20	20.00
> 15	17	17.00
Total	**100**	**100.00**

Majority (73%) of the patients had long standing diabetes (more than five years). The mean duration was 10.22 ± 7.00 years.

Table 4. Treatment of type 2 diabetes

Treatment	Distribution (n=100)	
	Number	**Percentage**
Oral	80	80.00
Insulin	20	20.00
Total	**100**	**100.00**

In the present study most of the subjects had long standing diabetes of more than 5 years (n=73). 80 % of these patients were on oral hypoglycemic drugs and remaining patients were on insulin.

Table 5. History of other co morbid illnesses

Other co morbid illnesses	Distribution (n=100)	
	Number	**Percentage**
Hypertension	77	77.00
Cerebrovascular accident	43	43.00
Cardiovascular disease	64	64.00
Diabetic Peripheral neuropathy	14	14.00
Diabetic retinopathy	46	46.00

The study population was screened for the presence of hypertension, cerebrovascular accidents, coronary artery disease, diabetic retinopathy and peripheral neuropathy. 77% (n=77) were hypertensive. 43% (n=43) patients reported a history of cerebrovascular accident. 64 patients had a significant past history suggestive of coronary artery disease. 46 patients had diabetic retinopathy and 14 were found to have diabetic peripheral neuropathy.

Table 6. Body mass index

Body mass index (Kg/m2)	Distribution (n=100)	
	Number	**Percentage**
< 18.5	0	0.00
18.5 - 24.99	1	1.00
25 - 29.99	53	53.00
> 30	46	46.00
Total	**100**	**100.00**

In the present study, 53 subjects were in the pre-obese group with a BMI between 25 to 29.99 kg/m^2 and 46 patients were obese with a BMI of > 30 kg/m^2. Only one patient had a normal BMI between 18.5-24.99 kg/m^2. The mean BMI of the study population was 29.69 ± 2.45 Kg/m^2.

Table 7. Waist circumference

Waist circumference	Distribution (n=100)	
	Number	**Percentage**
Normal	0	0.00
Abnormal	100	100.00
Total	**100**	**100.00**

In accordance with the IDF criteria for South Asians, waist circumference of ≥ 90 cms in males and > 80 cms in females is regarded as abnormal. All the subjects in the study had an abnormal waist circumference. The mean waist circumference for the study population was found to be 102.06 ± 5.75 cms.

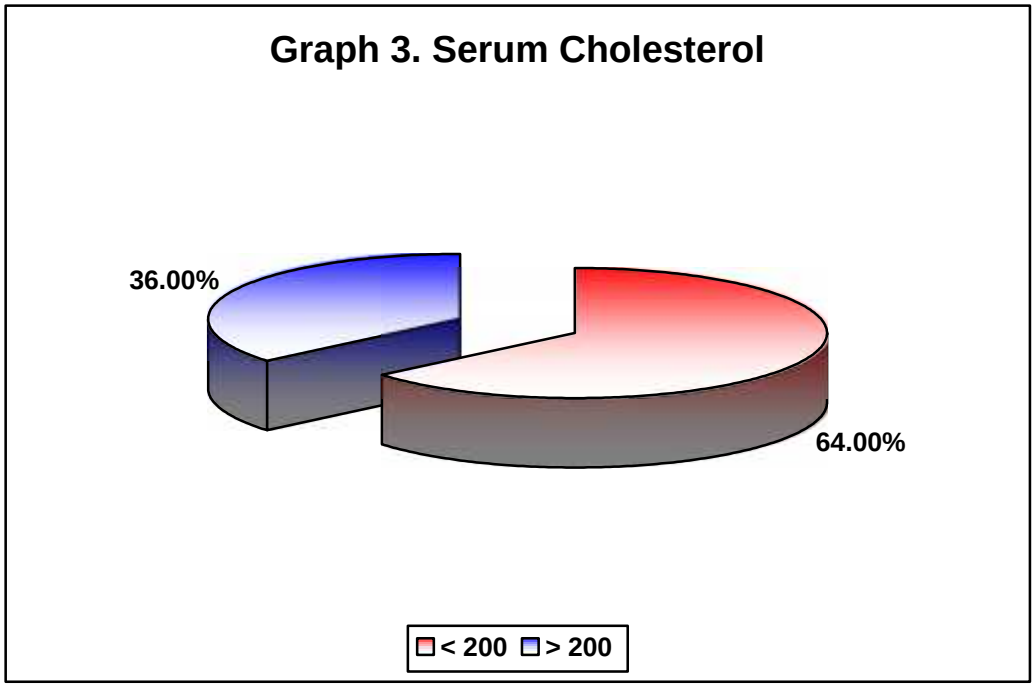
Table 8. Blood pressure

Parameters	Distribution (n=100)	
	Mean	**SD**
Systolic Blood pressure (mm Hg)	141.76	16.03
Diastolic blood pressure (mm Hg)	90.06	9.60

The mean blood pressure in this study was 141.76 ± 16.03 mm Hg systolic and 90.06 ± 9.60 mm Hg diastolic.

Table 9. Serum Cholesterol

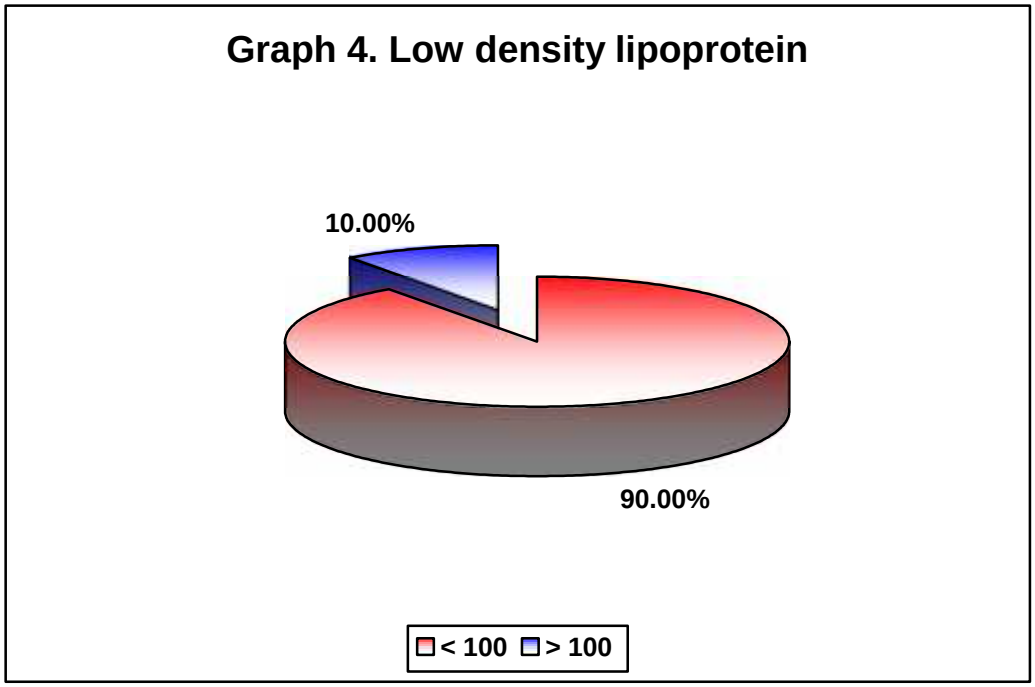
Serum cholesterol levels (mg/dL)	Distribution (n=100)	
	Number	**Percentage**
< 200	64	64.00
> 200	36	36.00
Total	**100**	**100.00**



In our study about 36 subjects had high serum cholesterol levels (more than 200mg/dl). The mean cholesterol level was 182.62 ± 38.21 mg/dL.

Table 10. Low density lipoprotein

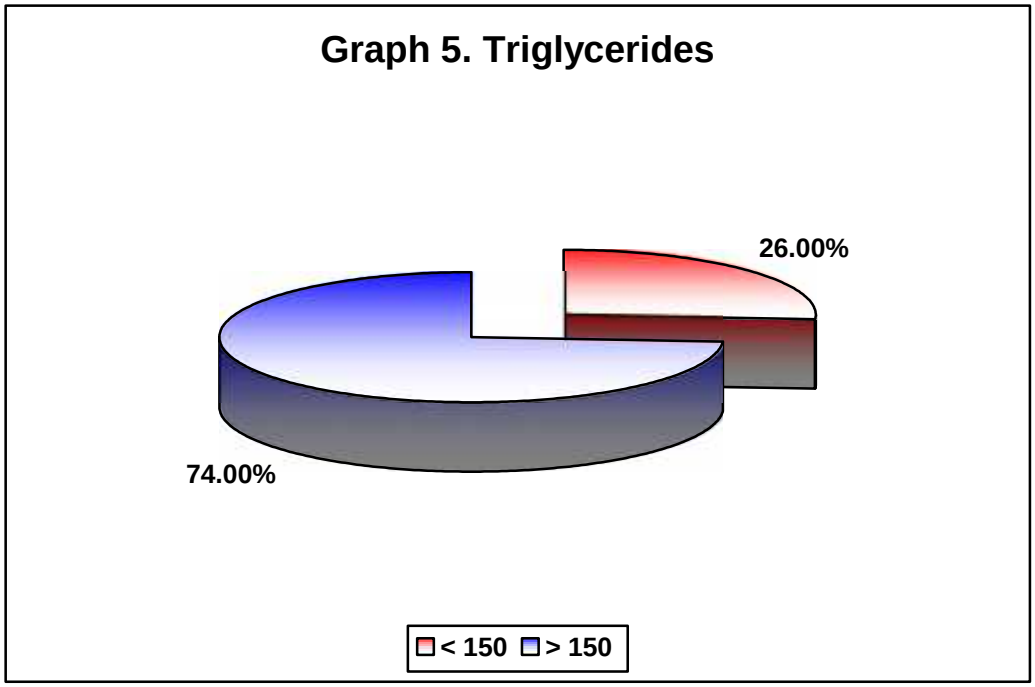
Low density lipoprotein (mg/dL)	Distribution (n=100)	
	Number	**Percentage**
< 100	90	90.00
> 100	10	10.00
Total	**100**	**100.00**



In our study, 90 percentage of the study population had high low density lipoprotein levels (more than 100 mg/dL). The mean LDL was 75.92 ± 20.66 mg/dL.

Table 11. Triglycerides

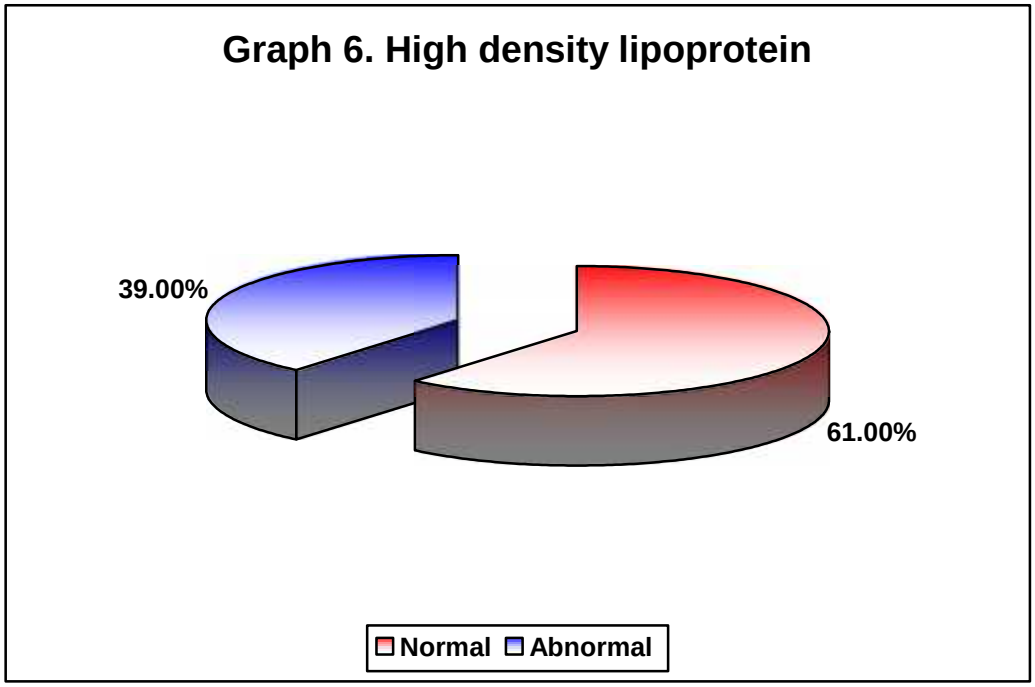
Triglyceride levels (mg/dL)	Distribution (n=100)	
	Number	**Percentage**
< 150	26	26.00
≥ 150	74	74.00
Total	**100**	**100.00**



In the present study, 74 subjects (74%) were found to have an abnormal TG, while the remaining 26 subjects had a Triglyceride level of < 150 mg/dL. The mean triglyceride level was 166.16 ± 40.91 mg/dL.

Table 12. High density lipoprotein

HDL Levels (mg/dL)	Distribution (n=100)	
	Number	**Percentage**
Normal	61	61.00
Abnormal	39	39.00
Total	**100**	**100.00**



In the present study, 39 subjects were found to have abnormal HDL levels whereas 61 subjects were found to be normal. The mean HDL level of this study was 41.49 ± 9.74 mg/dL.

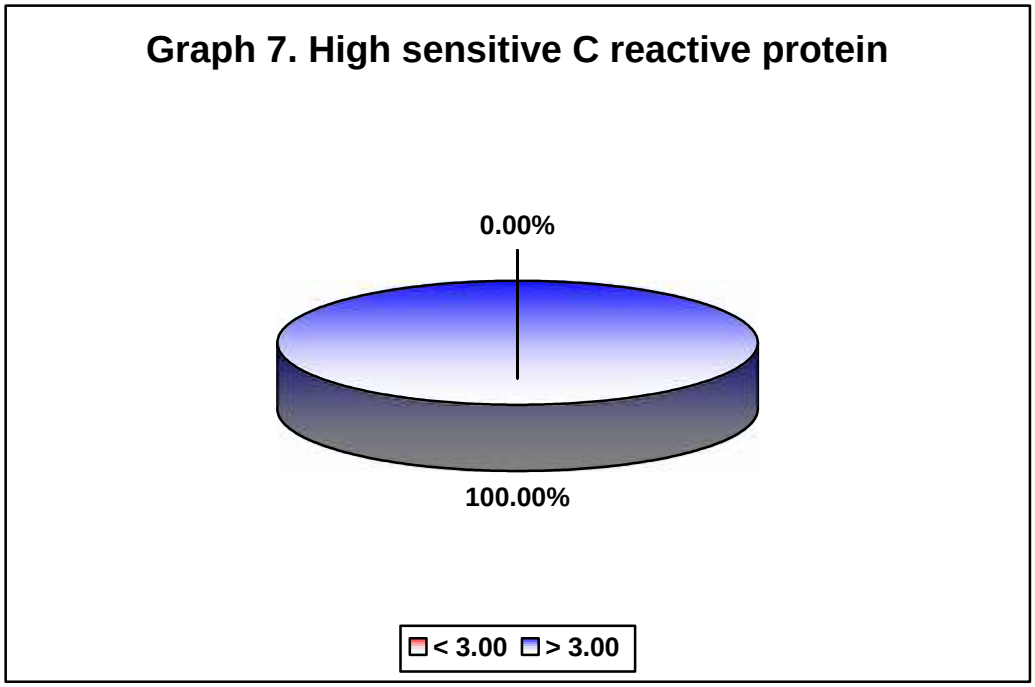
Table 13. HOMA-IR

HOMA-IR	Distribution (n=100)	
	Number	**Percentage**
< 3.80	0	0.00
≥ 3.80	100	100.00
Total	**100**	**100.00**

HOMA-IR was considered to be abnormal at levels in excess of 3.80. In the present study, all the patients had an elevated HOMA-IR indicating insulin resistance. The mean HOMA-IR level in this study was 15.53 ± 7.94.

Table 14. Hs-CRP

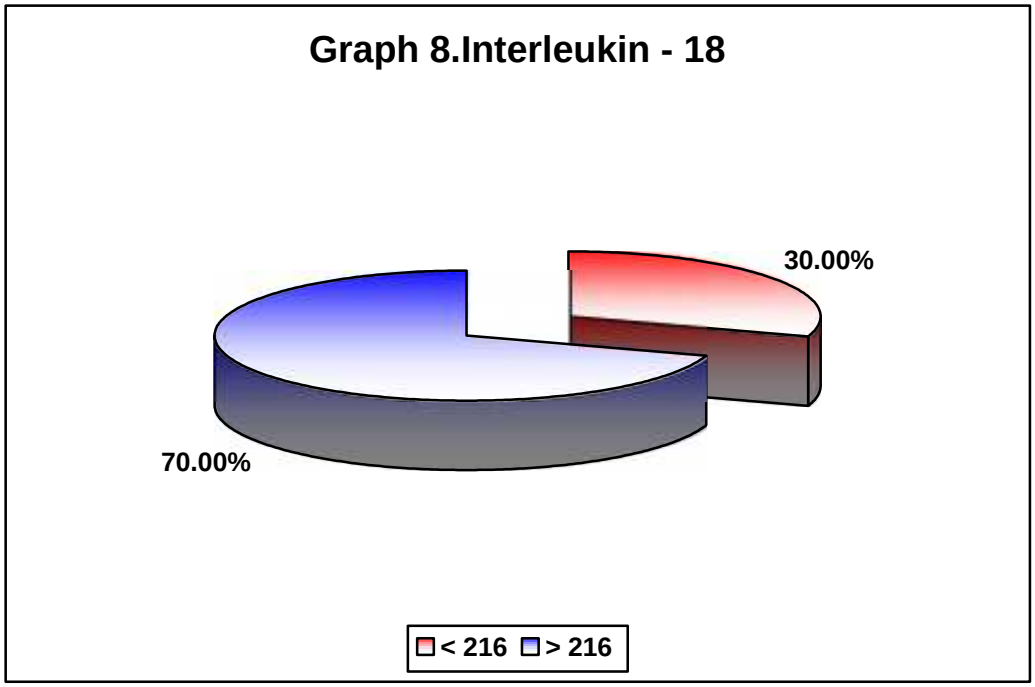
High sensitive C reactive protein (mg/L)	Distribution (n=100)	
	Number	Percentage
< 3.00	0	0.00
≥ 3.00	100	100.00
Total	**100**	**100.00**



Few studies have showed association between hs-CRP and diabetic nephropathy and have proposed it as an early predictor. A value in excess of 3 mg/L was considered as abnormal. In the present study, all the subjects had an abnormal hs-CRP with a mean of 19.96 ± 9.59 mg/L.

Table 15. Interleukin – 18

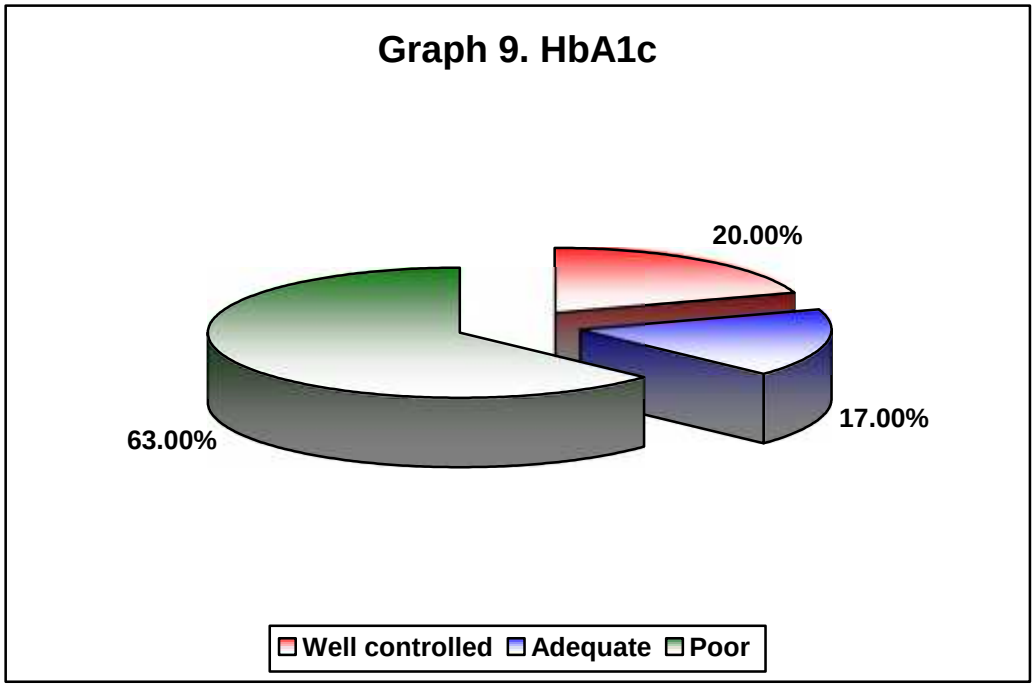
Interleukin - 18 levels (pg/mL)	Distribution (n=100)	
	Number	**Percentage**
< 216	30	30.00
≥ 216	70	70.00
Total	**100**	**100.00**



Using the standard MBL recombinant human IL-18 ELISA kit, values of ≥ 216 pg/mL was considered abnormal. In the present study, 70 patients (71%) were found to have an elevated IL-18 level, whereas 30 subjects had an IL 18 ≤ 215 pg/mL. The mean IL-18 level in this study was 260.89 ± 69.79 pg/mL.

Table 16. HbA1c

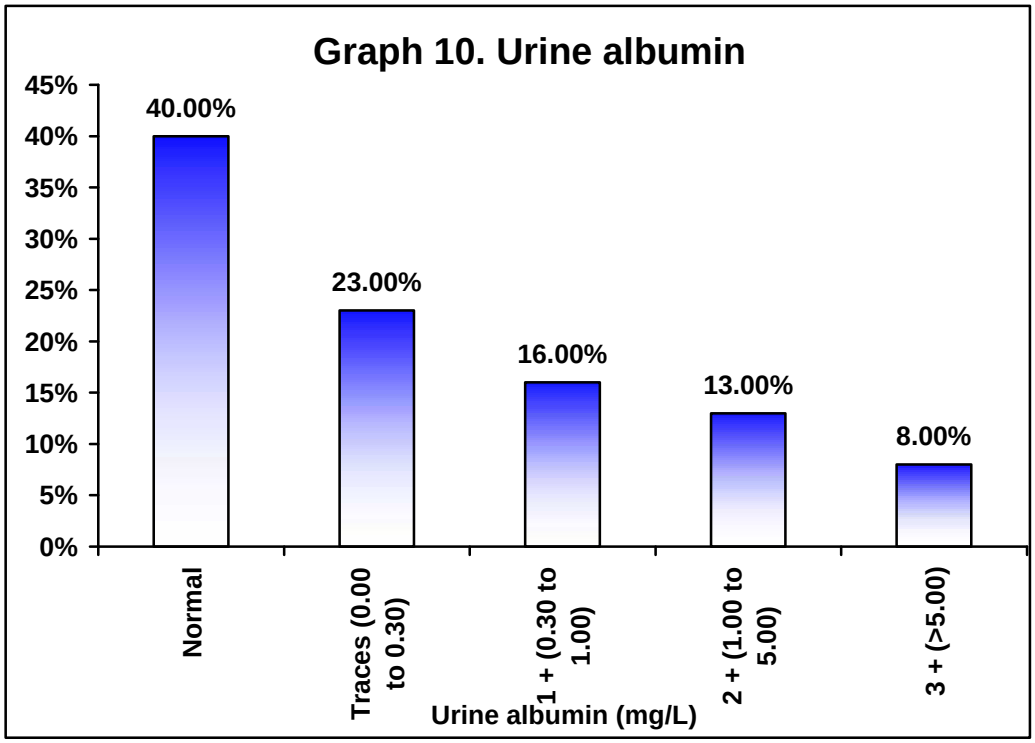
HbA1c Levels	Distribution (n=100)	
	Number	**Percentage**
Well controlled (<7.00)	20	20.00
Adequate (7.00 to 8.00)	17	17.00
Poor (> 8.00)	63	63.00
Total	**100**	**100.00**



HbA1c is a better measure for glycemic control over a period of 3 months and is gold standard for the same. In our study majority, about 63% of the subjects had poor glycemic control as demonstrated by the HbA1c levels of more than 8%. The mean HbA1c in the study population was 8.84 ± 1.98 percent.

Table 17. Urine Albumin

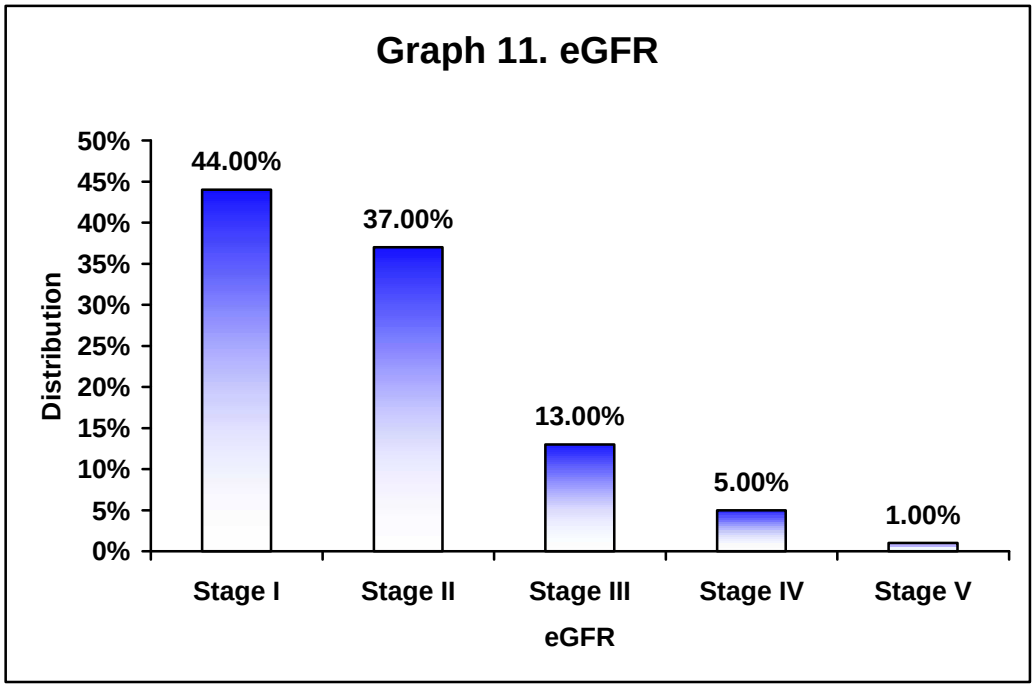
Urine albumin (gm/L)	Distribution (n=100)	
	Number	**Percentage**
Normal	40	40.00
Traces (0.00 to 0.30)	23	23.00
1+ (0.30 to 1.00)	16	16.00
2+ (1.00 to 5.00)	13	13.00
3+ (> 5.00)	8	8.00
Total	**100**	**100.00**



Urinary excretion of albumin is proved to be the early marker of diabetic nephropathy. In our study 6o % of the study population had urinary excretion of albumin ranging from traces to < 0.3 mg/dl, suggestive of microalbuminuria.

Table 18. eGFR

eGFR	Distribution (n=100)	
	Number	Percentage
Stage I	44	44.00
Stage II	37	37.00
Stage III	13	13.00
Stage IV	5	5.00
Stage V	1	1.00



Diabetic nephropathy is stratified into 5 stages using estimated GFR(calculated using MDRD formula) values. In our study, it was observed that 44% of the study population belonged to stage 1 and 37 % belonged to stage 2. The mean eGFR level was 89.31 ± 40.56 mL/min.

Table 19. Correlation of lipid profile with stages of kidney disease

Parameters		Stages									
		I (n=44)		II (n=37)		III (n=13)		IV (n=5)		V (n=1)	
		No	%	No	%	No	%	No	%	No	%
CHL	Normal	29	65.91	22	59.46	9	69.23	3	60	1	100
	abnormal	15	34.09	15	40.54	4	30.77	2	40	0	0.00
	Total	**44**	**100**	**37**	**100**	**13**	**100.00**	**5**	**100**	**1**	**100**
		Two way ANOVA between grade $F_{3,92}$=0.309; p=0.819									
		Two way ANOVA between Cholesterol $F_{1,92}$=45.459; p<0.001									
		Interaction $F_{3,93}$=0.373; p=0.772									
LDL	Normal	4	9.09	4	10.81	2	15.38	0	0	0	0.00
	abnormal	40	90.91	33	89.19	11	84.62	5	100	1	100
	Total	**44**	**100**	**37**	**100**	**13**	**100.00**	**5**	**100**	**1**	**100**
		Two way ANOVA between grade $F_{3,92}$=0.921; p=0.434									
		Two way ANOVA between LDL$F_{1,93}$=48.812; p<0.001									
		Interaction $F_{3,93}$=0.947; p=0.396									
HDL	Normal	26	59.09	20	54.05	10	76.92	5	100	0	0.00
	abnormal	18	40.91	17	45.95	3	23.08	0	0	1	100
	Total	**44**	**100**	**37**	**100**	**13**	**100.00**	**5**	**100**	**1**	**100**
		Two way ANOVA between grade $F_{3,92}$=2.595; p=0.057									
		Two way ANOVA between HDL $F_{1,92}$=59.638; p<0.001									
		Interaction $F_{3,92}$=1.358; p=0.261									
TGA	Normal	15	34.09	6	16.22	2	15.38	2	40	1	100
	abnormal	29	65.91	31	83.78	11	84.62	3	60	0	0.00
	Total	**44**	**100**	**37**	**100**	**13**	**100.00**	**5**	**100**	**1**	**100**
		Two way ANOVA between grade $F_{3,92}$=0.193; p=0.901									
		Two way ANOVA between TGA $F_{1,92}$=42.600; p<0.001									
		Interaction $F_{3,92}$=1.780; p=0.157									

In our study, the cholesterol values were found to be elevated in 36 patients (p=0.819). And the values increased as the diabetic nephropathy progressed with stages and this was statistically significant (p=0.001).

The LDL values were found to be high in 90% of the study population (p=0.021).

The HDL values were found to be low in 39 patients and normal in 61 patients, which was statistically not significant (p=0.057). Amongst the 39 patients, HDL was low in all stages of diabetic nephropathy (p<0.001).

The triglycerides were found to be abnormally high in 75 patients (p=0.90). Amongst the various stages of diabetic nephropathy, triglycerides were found to be high in each stage (p<0.001).

Table 20. Correlation of biomarkers with stages of kidney disease

Parameters		Stages									
		I (n=44)		II (n=37)		III (n=13)		IV (n=5)		V (n=1)	
		No	%	No	%	No	%	No	%	No	%
IL-18	Normal	13	29.55	11	29.73	4	30.77	2	40.00	0	0.00
	abnormal	31	70.45	26	70.27	9	69.23	3	60.00	1	100
	Total	**44**	**100**	**37**	**100**	**13**	**100**	**5**	**100**	**1**	**100**
		Two way ANOVA between grade $F_{3,92}$=0.810; p=0.492									
		Two way ANOVA between IL-18 $F_{1,92}$=42.600; p<0.001									
		Interaction $F_{3,92}$=0.248; p=0.863									
Hs-CRP	Normal	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	abnormal	44	100	37	100	13	100	5	100	1	100
	Total	**44**	**100**	**37**	**100**	**13**	**100**	**5**	**100**	**1**	**100**
		Two way ANOVA between grade $F_{3,92}$=0.799; p=0.497									

The inflammatory biomarkers were found to be elevated in majority of the patients. hsCRP was found to be high in all 100 patients. On further analysis according to stages of diabetic nephropathy, no stage wise association or any pattern was found (p=0.497).

IL-18 values were found to be high in 70 subjects (p=0.492). When analyzed according to stages of diabetic nephropathy it was found to be raised significantly, in all stages (p<0.001).

Table 21. Stages of diabetic nephropathy and mean lipid parameters

Parameters		Stages									
		I (n=44)		II (n=37)		III (n=13)		IV (n=5)		V (n=1)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CHL	Nor	159.3	28.9	164.6	30.3	168.3	28.8	154.0	9.0	190.0	0.0
	Abnormal	216.3	25.2	221.4	28.2	207.5	9.0	228.0	39.6	0.0	0.0
	Overall	178.75	38.7	187.6	40.6	180.4	30.5	184.0	45.2	190.0	0.0
LDL	Nor	110.3	6.9	121.8	18.4	105.0	1.4	0.0	0.0	0.0	0.0
	Abnormal	71.8	15.3	70.0	17.8	71.9	15.8	79.0	24.4	88.0	0.0
	Overall	75.3	18.4	75.6	24.0	77.0	19.0	79.0	24.4	88.0	0.0
HDL	Nor	122.4	27.7	113.2	33.6	94.5	72.8	143.0	4.2	146.0	0.0
	Abnormal	180.7	26.9	182.5	28.5	190.1	41.3	164.3	11.5	0.0	0.0
	Overall	160.8	38.8	171.2	38.8	175.4	56.1	155.8	14.4	146.0	0.0
TGA	Nor	34.8	6.1	35.9	5.0	38.1	6.5	31.4	7.7	0.0	0.0
	Abnormal	52.3	5.8	49.2	5.6	56.7	4.2	0.0	0.0	42.0	0.0
	Overall	41.9	10.5	42.0	8.5	42.4	10.1	31.4	7.7	42.0	0.0

Table 21 shows stages of diabetic nephropathy and mean serum cholesterol, low density lipoprotein, high density lipoprotein and triglycerides levels.

Table 22. Stage of kidney disease and mean biomarkers

Parameters		Stages									
		I (n=44)		II (n=37)		III (n=13)		IV (n=5)		V (n=1)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Hs-CRP	Nor	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Abnormal	18.3	8.4	21.6	10.0	20.4	11.4	18.0	9.7	38.0	0.0
	Overall	18.3	8.4	21.6	10.0	20.4	11.4	18.0	9.7	38.0	0.0
IL-18	Nor	180.5	10.3	189.0	16.6	186.0	19.7	195.5	7.8	0.0	0.0
	Abnormal	277.7	44.8	306.1	66.8	300.1	62.4	305.0	83.1	346.0	0.0
	Overall	249.0	58.7	271.3	78.2	265.0	75.5	261.2	84.1	346.0	0.0

Table 22 shows stages of diabetic nephropathy and mean hs-CRP and IL-18 levels. In all stages of diabetic nephropathy, mean hs-CRP and IL-18 levels were high.

Chapter 6

Discussion

DISCUSSION

Nephropathy associated with type 2 diabetes is a leading cause of end-stage renal disease. The earliest clinical sign of diabetic nephropathy is an elevated urinary AER, referred to as microalbuminuria. Microalbuminuria in diabetic patients has been recognized not only as a predictor of progression of diabetic nephropathy, but also as a powerful independent risk factor for cardiovascular disease. Epidemiological studies have demonstrated that only a limited proportion of diabetic patients develop microalbuminuria; thus, early identification of at-risk patients and initiation of appropriate therapy is important for the improvement of outcomes.[82]

In comparison to normoalbuminuric diabetic patients, those with microalbuminuria and overt proteinuria have been reported to have significantly higher plasma concentrations of very low density lipoprotein (VLDL), low density lipoprotein (LDL) and triglyceride but lower levels of high density lipoprotein cholesterol (HDL).[43] This study was taken up to prove the association of diabetic dyslipidemia with diabetic nephropathy.

Chronic subclinical inflammation has been proposed to be involved in the development of microalbuminuria in diabetes. This hypothesis is supported by several cross-sectional studies reporting elevated levels of markers of inflammation, such as high-sensitivity C-reactive protein (hs-CRP), TNF-α and IL-1, in diabetic patients with diabetic nephropathy versus those without.[82] Hence inflammatory biomarkers were also studied in this study to see association between the two.

The present study, was undertaken to find out the association of inflammatory biomarkers (IL-18, hs-CRP) and lipid profile in type 2 diabetes mellitus with or without diabetic nephropathy.

This one year cross sectional study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on patients with type 2 diabetes mellitus. A total of 100 patients with type diabetes mellitus were selected for the study.

In the present study, 100 patients were subjected for detailed examination like blood pressure, body mass index, waist hip ratio and other systemic examination.

The study population was thoroughly investigated for glycemic control, lipid status and diabetic nephropathy profile and also for inflammatory biomarkers like Interleukin -18, hsCRP.

In the present study, males outnumbered (64%) females (36%) with male to female ratio of 1.77:1. In a study by araki et al, males constituted 60 % (n=150) patients of the total 250 diabetic patients in whom progression of diabetic nephropathy was studied.

Majority of the subjects were between 61 and 70 years accounting for 38% (n=38), with 15 and 37 subjects between 41-50 and 51-60 respectively. Only a small number were below 40 or above 70 years (n=1, n=1 respectively). The mean age for the study population was 60.3 ± 9.43 years. In a similar study done

In a study[82] the mean age of the study population was 61±9 years. In another study[10] the mean age of study population was 65.5±7.9 years.

In the present study majority 73% (n=73) of the patients had long standing diabetes (more than five years), 80% (n=80) of patients were on oral hypoglycemic drugs. Overall, the mean duration was 10.22 ± 7.00 years. A study[82] observed that duration of diabetes in their study population was 13±8 years. In another study[10] the mean duration of diabetes in study population was 11.3±9.2years (0.1–43.8)

Co-morbid states in these patients included hypertension (77%), cerebrovascular accident (43%) coronary artery disease (64%). In our study population it was observed that 46% patients suffered from diabetic retinopathy and 14% of patients had diabetic peripheral neuropathy. Similar results were found in the study[10] which reported 76% of patients (182/250) were observed to be suffering from diabetic retinopathy.

In the present study, more than half (53%) were overweight (BMI 25 to 29.99 kg/m^2) and 46% were obese (BMI > 30 kg/m^2). Overall, the mean BMI of the study population was 29.69 ± 2.45 Kg/m^2. This was high when compared with a study[82] in Japanese population, in who mean BMI was 23±3years. Where as in a study[10] in Saudi Arabian population, mean BMI was 27.8±4.4 (18.3–56.5) kg/m^2.

All the subjects in the study had an abnormal waist circumference based on the IDF criteria. The mean waist circumference for the study population was

found to be 102.06 ± 5.75 cms. This was significantly high when compared to study[82] population of, in who mean waist circumference was 88±9 cms.

The mean blood pressure in this study was 141.76 ± 16.03 mm Hg systolic and 90.06 ± 9.60 mm Hg diastolic. This was comparable to the average blood pressure in study[82] where it was134±15 mm Hg systolic and 138±16 mm hg Diastolic. In a study[83] IL-18 was studied as a predictor of diabetic nephropathy wherein the authors reported the mean blood pressure as 124 ± 14 mm Hg systolic and 75 ±10 mm Hg diastolic.

In our study, we observed 36% (n=36) patients had high serum cholesterol levels(more than 200mg/dl), The mean cholesterol level was 182.62 ± 38.21 mg/dL. This was comparable to Saudi Arabian population study[10] where the mean cholesterol level was 200 mg/dl. Multiple studies[82,83] on Japanese population also observed high serum cholesterol levels 100.6±14.2 mmol/l and 99.3±15 mmol/l respectively. Another study[84] observed the mean cholesterol 190.5±15.6 mg/dl. This value was raised and was statistically significantly associated with diabetic nephropathy. Previous studies[82,83,84] carried out have proved that increased serum cholesterol levels are associated with progression of diabetic nephropathy. In our study, the cholesterol values were found to be elevated in 36 patients (p=0.819) and the values increased as the diabetic nephropathy progressed with stages and was statistically significant (p=0.001).

It was observed that LDL values were found to be high in 90% of the study population (p=0.021) and the mean LDL was 75.92 ± 20.66 mg/dL. This was in contrast to Saudi Arabian population study[10] where the mean LDL level

was 123 mg/dl. The two Japanese studies[82,83] also observed high LDL levels 72.36±14.22 mmol/l and 59.58±15.22 mmol/l respectively. Another study[84] observed that mean LDL value was 146.88±11.88 which was raised and statistically significantly associated with diabetic nephropathy. Previously published studies[10,82-84] on the association between LDL and presence of kidney disease have yielded ambiguous results, with a couple of investigations reporting a positive association, and others reporting no association

In the present study, HDL values were found to be low in 39 patients and normal in 61 patients, which was statistically not significant (p=0.057) and mean HDL level was 41.49 ± 9.74 mg/dL. In all the stages of diabetic nephropathy in these patients it was noted that HDL was low (p<0.001). This was similar to Saudi Arabian population study[10] where the mean HDL level was 39mg/dl and they noted that lower HDL levels were more common in those with moderate renal insufficiency, defined as GFR < 60ml/min/1.73 m2. The study[82,83] on Japanese population also observed low HDL levels 26.46±64.8 mmol/l and 28.98±7.92 mmol/l respectively. Another study[84] observed that mean HDL values were 41.87±2.87 mg/dl and this value was raised and was statistically significantly associated with diabetic nephropathy.

This observation is consistent with previous investigations demonstrating that those with kidney dysfunction have 11% to 32% lower HDL levels.

Diabetic dyslipidemia is associated characteristically with increased Triglyceride levels. In our study, the triglycerides were found to be abnormally High in 75 patients (p=0.90). Amongst each stage of diabetic nephropathy

Triglycerides were found to be high in each stage ($p<0.001$). The mean Triglyceride level was 166.16 ± 40.91 mg/dL. This was similar to Saudi Arabian Population study by lin et al, where the mean TG level was 157 mg/dl and they Noted that higher TG levels were more common in those with moderate renal Insufficiency, defined as GFR < 60ml/min/1.73 m2. A study[82] also observed High TG levels 1.06±0.41 mmol/l. Another study[84] observed that Mean TG value was 177.88±88.67 which was raised and statistically significantly associated with diabetic nephropathy.

Insulin resistance is a central feature of type 2 diabetes mellitus. There are various measures of insulin resistance and HOMA-IR was used in this study. HOMA-IR was considered to be abnormal at levels in excess of 3.80. In the present study, all the patients had an elevated HOMA-IR indicating insulin resistance. The mean HOMA-IR was 15.53 ± 7.94. This is one of the few studies to consider HOMA-IR as the insulin sensitivity model.

The pathogenesis of diabetic nephropathy is related to chronic hyperglycemia. The mechanisms by which chronic hyperglycemia leads to diabetic nephropathy, involve the effects of soluble factors (growth factors, angiotensin II, endothelin, AGEs), hemodynamic alterations in the renal microcirculation (glomerular hyperfiltration or hyperperfusion, increased glomerular capillary pressure), and structural changes in the glomerulus (increased extracellular matrix, basement membrane thickening, mesangial expansion, fibrosis). Glycated hemoglobin is proven measure of glycemic control in diabetic patients. In the present study majority, about 63% of the patients had poor glycemic control as demonstrated by the HbA1c levels of more than 8%.

The mean HbA1c in the study population was 8.84 ± 1.98 percent. In their study nakamura et al observed the mean HbA1c was 7.3±1.1 and in a study[82] it was 7.5±0.9.

In the past three decades, urinary albumin excretion has assumed a central role in the diagnosis and management of kidney disease among people with diabetes. Microalbuminuria was initially found to predict subsequent overt albuminuria (more than 300mg/24h), which in turn predicted loss of GFR. From the strength of these relationships, it has frequently been assumed that microalbuminuria and overt albuminuria are requisite first and second steps along a single pathway that leads to loss of GFR and ESRD. Persistent microalbuminuria was strong risk factor for subsequent loss of GFR, reemphasizing earlier work that established the importance of sustained increases in urine albumin excretion in the pathogenesis and diagnosis of diabetic nephropathy. In this study 60% of the patients had urinary excretion of albumin ranging from traces to >5 mg/dl. This was similar to the study[82] where authors observed that mean AER was 0.0487 mg/min which was raised and was statistically significant. Also another study[83] noted that AER was 103 ± 432 mg/gCr and this value was statistically significant.

Diabetic nephropathy is stratified into five stages based on estimated GFR Calculated using MDRD formula. It was observed that 44% of the study Population belonged to stage 1 and 37 % belonged to stage 2. The mean eGFR Level was 89.31 ± 40.56 mL/min. This was similar to the study[82] where the mean eGFR (min ml^{-}1/1.73 m2) was 104±21 min ml^{-}1/1.73 m^{2}. Highly sensitive C-reactive protein is proven as a marker of inflammation by various previous

studies. In the present study, all the subjects had an abnormal hs-CRP with a mean of 19.96 ± 9.59 mg/L. Similarly it was reported to be high in another study[83] 1.05±1.43 mg/l without statistical significance. Also another study[82] noted similar results i.e. raised hsCRP levels but without statistical significance. In our study, it was observed that this biomarker of inflammation was raised in all stages of diabetic nephropathy. On further analysis of this marker, no stage wise increase in the mean values was observed in our study.

Interleukin-18, a recently described member of the IL-1 cytokine super family, is now recognized as an important regulator of innate and acquired immune responses. IL-18 is expressed at sites of chronic inflammation, in autoimmune diseases, in a variety of cancers, and in the context of numerous infectious diseases and in 71% of the patients IL-18 level was elevated. Overall, the mean IL-18 level in this study was 260.89 ± 69.79 pg/mL. In a study[82] it was observed that IL-18 was raised i.e. 154 ng/l in majority of the patients and was statistically significant. Similarly another study[83] also it was observed that mean IL-18 levels in their study was 179 ±63 and this value was statistically significant. Similarly other study[84] observed that, mean IL-18 value was 115.96±89.8 pg/ml and this value was raised and was statistically significantly associated with diabetic nephropathy.

In our study the cholesterol and LDL values were found to be significantly elevated in patients with diabetic nephropathy and the values increased as the diabetic nephropathy progressed ($p<0.05$). Whereas, the HDL was found to be low in 39 patients and normal in 61 patients with no statistically significant difference being observed ($p=0.057$). However, amongst the stages of

diabetic nephropathy, HDL was low in all stages ($p<0.001$). The triglycerides were found to be abnormally high in 75 patients ($p=0.90$). Amongst the stages of diabetic nephropathy, Triglycerides were found to be high in each stage ($p<0.001$).

The inflammatory biomarkers were found to be elevated in majority of the patients. hsCRP was found to be high in all 100 patients. On further analysis according to stages of diabetic nephropathy no stage wise association or any pattern was found ($p=0.497$).

IL-18 values were found to be high in 70 subjects ($p=0.492$). It was found to be statistically significantly raised in all stages of diabetic nephropathy ($p<0.001$).

Chapter 7

Conclusion

CONCLUSION

- Serum cholesterol and low density lipoprotein values were found to be significantly elevated in patients with diabetic nephropathy and the values increased as the diabetic nephropathy progressed.

- High density lipoprotein was found to be low in many patients but no association was observed with stages of diabetic nephropathy.

- Triglycerides were found to be significantly high in most patients and were high amongst the various stages of diabetic nephropathy.

- hsCRP was found to be high in all patients of diabetic nephropathy. There was no definite pattern of rise or stage wise association with stages of diabetic nephropathy.

- IL-18 was found to be high in most of the patients of diabetic nephropathy. There was no definite pattern of rise or stage wise association with stages of diabetic nephropathy.

Chapter 8

Summary

SUMMARY

Type 2 DM is now recognized as an inflammatory condition associated with insulin resistance and abnormal endothelial vascular reactivity. Dyslipidemia and inflammation may promote renal disease via mechanisms of vascular endothelial cell dysfunction in type 2 diabetes mellitus (DM). Several potentially modifiable lipid and inflammatory biomarkers are elevated in the setting of moderately decreased GFR in men with type 2 DM. The present study was undertaken to find out the association of inflammatory biomarkers (IL-18, hs-CRP) and lipid profile in type 2 diabetes mellitus with or without diabetic nephropathy, thus may reveal new approaches to the prevention of progressive renal insufficiency.

The present one year cross sectional study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. A total of 100 patients with type diabetes mellitus were selected for the study. Based on the simple random sampling where every third patient who fulfilled the selection criteria was included in the study.

In the present study, males outnumbered (64%) females (36%) with male to female ratio of 1.77:1. Majority of the subjects (38%) were aged between 61 and 70 years with the mean age was 60.3 ± 9.43 years. All the subjects had an abnormal hs-CRP with a mean of 19.96 ± 9.59 mg/L and in 71% of the patients IL-18 level were elevated. Overall, the mean IL-18 level in this study was 260.89 ± 69.79 pg/mL. 60% of the patients had urinary excretion of albumin ranging from traces to >5 mg/dL. It was observed that 44% of the study population

belonged to stage 1. The mean eGFR level was 89.31 ± 40.56 mL/min. The lipid abnormalities and inflammatory biomarkers (IL-18, hsCRP) were found to be significantly high in patients with diabetic nephropathy.

Findings of this study indicate an association of diabetic dyslipidemia and chronic inflammation with the pathogenesis of diabetic nephropathy. Hence inflammatory biomarkers (IL-18, hsCRP) can be used as an early screening test for diabetic nephropathy. The possibility of IL-18 as a therapeutic target needs to be explored in further studies.

Chapter 9

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Annexures

Annexure I

ANNEXURE I – CONSENT FORM

"A STUDY OF ASSOCIATION OF INFLAMMATORY BIOMARKERS (IL-18,hSCRP) AND SERUM LIPIDS IN TYPE 2 DIABETES MELLITUS PATIENTS WITH OR WITHOUT DIABETIC NEPHROPATHY"

Objective and purpose of the study:

This research is intended to study the association of serum lipids and inflammatory biomarkers (hs-CRP, IL-18) with renal function in patients with type-2 diabetes mellitus. The principal investigator of the study is Dr. **** *** under the guidance of Dr. **** ****. My co-operation will be of great help to patients with type-2 diabetes mellitus, dyslipidemias and also predict their complications in the future.

Procedure

If you agree to be part of the research study you will be asked the relevant history and will be subjected to relevant clinical examination and investigations. You will also have to give blood and urine samples for the necessary investigations

Risk and Benefits

The only risk and possible discomfort you might get is while taking blood from your arm for the investigations. It may cause swelling, pain, redness, bruising or infection (rarely happens) at the site from where the blood is drawn.

Alternatives

Taking part in this study is voluntary. You may choose not to take part in this study, or if you decide to take part now, you can later change your mind and withdraw from the study. Your decision will not change the present or future health care or other services that you receive. The study doctor or sponsor may you're your participation in this study any time. If you choose not to take part in the study you will receive the standard treatment for patients with your condition.

PRIVACY AND CONFIDENTIALITY

All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study may be published but your identity will be confidential in any publication.

Institution / Sponsor's policy

Does not apply to this research

Financial incentives for participation

You will not be paid / offered any gifts /incentives for participating in the study.

Authorization to publish the results

The results of the study would be forwarded to the KLE University, Belgaum as part of requirement towards the completion of MS degree, review and publishing.

If I have any questions about your rights as a participant you may call Principal and Chairman, J.N.M.C Ethical Committee for Human Research.

Consent Statement

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

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

Principal investigator : Dr. **** ***

Guide : Dr. *** *****

Name of the Participant: _____ Signature / Thumb print

Name of the Witness _____ Signature _____

Name of the investigator _____ Signature _____

Date: Place:

Annexures

Annexure II

ANNEXURE II – PROFORMA

Case No:

NAME: AGE/SEX:

IP No. ADDRESS:

OCCUPATION:

COMPLAINTS AT PRESENTATION:

HISTORY PERTAINING TO TYPE 2 DIABETES MELLITUS :

- k/c/o Type 2 diabetes mellitus yes ☐ no ☐

  If yes,

  Duration of illness -

  Details of treatment –

  Past history of cerebrovascular events –

  Past history of angina pain/ Myocardial infarction/ Ischaemic heart disease or on any specific medication.

  H/o peripheral neuropathy - Yes ☐ No ☐

  Documented Diabetic retinopathy/surgical management of retinopathy in the past

  Diabetic nephropathy Yes ☐ No ☐

  Diabetic foot Yes ☐ No ☐

  Autonomic neuropathy Yes ☐ No ☐

- K/c/o Hypertension or on specific anti-hypertensive medication

  Yes ☐ No ☐

- H/o Peripheral vasucular disease/past h/o treatment for the same

  Yes ☐ No ☐

- K/c/o of dyslipidemia or on any specific medication

Yes ☐ No ☐

- ON EXAMINATION :
- NUTRITION :
- HEIGHT :
- WEIGHT:

BMI :

- WAIST CIRCUMFERENCE :
- BLOOD PRESSURE :
- PULSE RATE:

SYSTEMIC EXAMINATION:

- RESPIRATORY SYSTEM:
- CARDIOVASCULAR SYSTEM:
- PER ABDOMEN:
- CENTRAL NERVOUS SYSTEM:

INVESTIGATIONS :

- FBS –
- TOTAL TRIGLYCERIDES –
- TOTAL HDL –
- TOTAL CHOLESTEROL-
- ESTIMATED LDL -
- HsCRP LEVELS –
- INTERLEUKIN 18 LEVELS –
- SERUM CREATININE-
- HEMOGLOBIN A1c-

ANNEXURE III - MASTER CHART

Serial Number	In Patient Number	Age (Years)	Sex	Duration Type 2 DM (years)	Treatment	Hypertension	Cerebrovascular accidents	Cardiovascular disease	Peripheral neuropathy	Diabetic retinopathy	Body Mass Index (Kg/m2)	Waist circumference	Fasting blood sugar (mg/dL)	Total Cholesterol (mg/dL)	Low density lipoprotein (mg/dL)	Triglycerides (mg/dL)	High density lipoprotein (mg/dL	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg	Fasting insulin levels	HOMA IR	Hs-CRP (mg/L)	Interleukin 18 (pg/mL)	Serum Creatinine (mg/dL)	HbA1c (%)	Urine albumin (gm/L)	eGFR
1	402311	58	M	3	O	+	-	-	-	-	27.89	95	116	165	36	146	18	138	90	31.0	12.50	11.1	201	4.0	8.9	T	16
2	402505	55	M	7	O	+	-	-	-	+	29.30	101	160	100	46	180	38	150	90	36.0	8.80	14.0	218	1.2	9.8	T	67
3	403376	65	M	15	O	+	-	+	-	+	30.82	98	144	110	36	214	31	140	90	39.0	10.49	17.1	252	1.2	7.8	-	65
4	403454	60	M	10	O	-	-	+	-	+	26.18	96	106	151	62	223	48	110	70	17.0	6.27	6.0	194	0.8	8.6	T	105
5	405981	60	F	2	O	+	+	-	-	-	30.89	98	176	150	54	202	24	156	90	36.8	13.49	16.0	246	0.6	6.4	T	108
6	405656	62	F	12	O	+	+	+	-	-	27.53	100	148	203	58	264	34	142	90	34.8	17.27	17.0	258	0.9	10	T	67
7	405493	58	M	6	O	+	+	-	-	-	24.67	94	98	160	36	154	52	158	100	22.0	8.60	8.0	196	1.0	8.4	-	82
8	405589	49	F	2	O	-	-	+	+	+	28.15	101	190	192	76	242	44	126	80	31.0	14.55	14.0	208	1.4	6.8	-	42
9	405816	50	M	4	O	+	+	+	-	+	30.42	106	204	106	53	91	35	154	90	40.0	10.36	21.0	294	0.8	10	T	109
10	404558	52	M	8	O	+	-	+	-	+	30.97	104	138	115	67	96	30	140	90	34.0	9.56	8.1	230	0.8	7.6	-	108
11	405254	45	F	1	O	+	-	-	-	-	26.29	90	110	132	46	160	52	140	90	20.0	6.45	10.0	190	0.6	6.4	-	115
12	404208	82	F	30	I	+	+	+	-	+	27.34	98	118	100	51	43	39	130	80	31.0	7.58	14.0	248	1.4	5.8	T	38
13	403730	76	M	15	I	+	+	+	-	-	30.45	102	176	150	90	153	35	138	90	28.0	10.27	16.0	256	2.7	8.5	1	25
14	404899	65	M	1	O	+	-	-	-	-	31.04	98	120	149	84	164	32	144	100	14.0	5.10	7.1	190	4.2	8.6	4	15
15	403026	64	M	10	O	+	+	+	-	+	31.77	110	216	200	80	192	26	150	100	38.0	18.58	20.0	277	0.6	9.7	1	144
16	405379	32	F	2	O	+	-	+	+	-	29.13	104	145	190	64	155	52	130	80	29.0	13.47	16.0	218	0.7	9.8	T	103
17	403847	59	M	15	O	+	-	-	-	+	30.01	106	140	150	70	165	40	156	90	24.0	8.80	12.0	196	1.1	8.8	-	73
18	405589	65	M	20	O	-	+	+	-	-	27.28	101	114	180	68	180	54	120	90	34.0	14.96	10.0	242	1.0	6.4	1	80
19	407599	62	F	6	O	+	+	+	+	+	33.20	120	230	170	90	233	30	138	100	38.0	15.79	22.0	306	0.5	10	1	133
20	408176	65	F	1	O	+	-	+	-	-	29.58	98	120	171	92	203	58	150	90	26.0	10.87	18.0	194	1.1	9.6	-	53
21	407746	60	F	2	O	+	-	-	-	-	28.47	96	88	294	95	195	59	150	90	31.0	22.28	20.0	232	0.5	6.6	-	134
22	408393	65	M	10	O	-	+	+	-	+	25.34	100	144	109	41	119	34	120	80	37.6	10.02	10.0	244	0.4	7.1	-	229
23	409483	48	F	5	O	+	-	+	-	-	25.91	101	124	202	104	282	30	140	90	56.0	27.65	12.0	316	1.2	8.2	1	51
24	409468	52	M	6	O	-	-	-	-	-	25.34	102	140	130	68	170	54	120	80	24.0	7.63	10.0	196	1.1	6.5	2	75
25	409548	52	M	10	I	+	-	+	-	-	29.30	109	162	197	59	185	26	140	90	33.2	15.99	14.0	238	0.5	9.4	T	186
26	410574	62	F	5	O	+	-	-	-	+	29.40	96	150	202	105	251	44	156	90	21.0	10.37	16.0	186	0.7	7.3	1	90
27	411261	69	M	12	O	+	+	+	-	-	30.44	98	126	150	97	154	30	160	90	38.0	13.93	18.0	296	0.6	6.6	-	142
28	411268	64	M	30	I	+	-	+	-	-	28.70	101	190	127	74	160	57	140	90	39.1	12.14	15.0	301	0.9	12	-	90
29	411810	55	M	6	O	+	-	-	-	+	30.40	110	128	124	68	75	41	136	86	24.0	7.27	16.0	198	1.0	13	T	82
30	411914	62	M	10	O	-	-	-	-	+	30.00	106	148	200	78	140	39	120	80	18.0	8.80	10.0	176	0.8	7.8	T	104
31	410767	60	M	8	O	-	+	+	-	+	30.48	108	126	190	56	71	36	110	70	30.0	13.93	12.0	219	0.5	13	-	180
32	412091	78	F	8	O	+	-	-	-	+	31.90	112	177	141	68	76	56	140	90	11.1	3.83	22.0	174	0.8	10	T	75
33	411941	48	M	5	O	-	+	+	-	+	27.30	98	186	221	120	208	44	120	80	40.0	21.61	21.0	320	0.9	14	1	96
34	412143	64	F	10	O	+	-	-	-	-	26.80	92	120	110	54	78	38	136	90	16.0	4.30	10.0	176	0.5	12	2	132
35	409488	71	M	20	I	+	-	+	-	-	29.06	101	106	203	38	160	48	138	100	26.0	12.90	13.0	218	1.1	6.6	T	70
36	409581	53	M	6	O	+	-	-	-	-	30.10	104	96	180	62	140	44	150	100	22.0	9.68	10.0	174	1.2	7.5	-	67
37	409781	64	F	10	O	+	+	+	+	+	33.70	118	164	203	64	200	30	160	100	56.0	27.79	28.0	400	0.6	8.6	-	107
38	409774	59	M	5	O	+	-	+	-	-	28.48	100	110	144	54	169	32	150	90	32.0	11.26	10.0	206	1.0	8	-	81
39	409929	55	M	3	O	-	-	-	-	-	27.04	98	136	176	70	100	34	110	70	20.0	8.60	8.0	166	0.7	6.5	-	124
40	409844	50	M	3	O	-	-	-	+	+	28.30	96	120	190	70	155	52	120	70	18.0	8.36	10.0	160	1.0	7.4	-	84
41	402201	44	F	5	O	+	-	+	-	-	30.17	104	112	184	76	180	34	150	100	34.0	15.29	16.0	284	0.6	6.8	-	115
42	402693	65	F	10	O	-	+	+	-	+	32.04	108	150	190	58	264	34	120	80	44.0	20.44	26.0	360	0.7	8.4	1	89
43	402593	70	M	20	I	+	-	+	-	+	27.45	103	124	140	50	190	55	140	90	38.0	13.00	24.0	219	1.0	9.6	-	79
44	402876	71	F	25	I	+	+	+	-	+	29.74	108	148	208	80	200	38	160	100	48.0	24.41	23.0	392	1.2	9.4	4	47
45	403171	43	M	1	O	+	-	-	-	-	29.92	98	118	130	60	156	48	150	90	22.0	6.99	16.0	194	0.9	6.8	-	98
46	403106	69	F	16	I	+	+	+	+	+	30.48	101	200	220	100	190	30	170	100	54.0	29.04	32.0	456	0.9	10	T	66
47	402834	51	M	5	O	-	-	+	+	+	38.37	106	155	225	90	210	23	120	70	39.0	21.45	20.0	301	1.0	7.5	T	84
48	403978	46	F	7	O	+	-	-	-	-	30.30	101	118	210	100	140	38	160	100	18.0	9.24	6.0	178	0.5	6.9	2	141
49	404210	64	M	10	O	-	+	-	+	+	26.58	98	156	196	56	154	60	120	70	36.0	17.25	18.0	238	1.9	12	-	38
50	404481	60	F	12	I	+	+	+	-	-	30.40	102	120	200	84	160	38	170	100	40.0	19.56	22.0	296	0.8	9.6	T	78
51	404375	61	M	15	O	+	-	+	+	+	29.71	101	138	166	88	155	55	140	90	38.0	15.42	19.0	277	0.8	8.2	-	104
52	404417	58	M	6	O	+	+	+	-	-	29.60	102	122	198	90	100	38	160	100	39.0	18.88	28.0	310	1.2	8.4	-	66
53	403552	55	M	4	O	-	-	-	-	+	28.12	104	140	244	66	155	56	110	70	21.0	12.53	8.0	168	0.7	7.5	-	124
54	405016	57	M	10	O	+	-	+	-	-	26.50	100	120	204	80	170	34	160	100	40.0	19.95	26.0	290	1.0	9.4	-	82
55	405981	60	F	16	O	+	+	+	-	+	32.13	104	210	230	86	204	32	150	90	46.0	25.86	30.0	362	0.7	6.3	T	91
56	405671	42	F	2	O	-	-	-	-	-	27.02	96	136	134	60	156	60	120	80	180.0	58.96	8.0	170	0.6	8.2	-	117
57	412997	70	M	15	O	+	-	+	-	+	30.30	106	148	200	78	168	44	140	90	36.0	17.60	24.0	262	1.0	8	2	79
58	413726	65	F	10	O	+	+	+	-	-	31.80	102	166	198	64	188	44	150	100	40.0	19.36	31.0	308	0.7	7.2	1	89

ANNEXURE III - MASTER CHART

Serial Number	In Patient Number	Age (Years)	Sex	Duration Type 2 DM (years)	Treatment	Hypertension	Cerebrovascular accidents	Cardiovascular disease	Peripheral neuropathy	Diabetic retinopathy	Body Mass Index (Kg/m2)	Waist circumference	Fasting blood sugar (mg/dL)	Total Cholesterol (mg/dL)	Low density lipoprotein (mg/dL)	Triglycerides (mg/dL)	High density lipoprotein (mg/dL	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg	Fasting insulin levels	HOMA IR	Hs-CRP (mg/L)	Interleukin 18 (pg/mL)	Serum Creatinine (mg/dL)	HbA1c (%)	Urine albumin (gm/L)	eGFR
59	413721	56	M	6	O	-	+	+	-	-	29.80	104	144	218	90	166	34	120	80	48.0	25.58	20.0	388	1.2	8.4	-	67
60	413874	60	M	10	O	+	-	+	-	-	32.80	112	108	192	76	172	56	150	100	36.0	16.90	22.0	290	0.9	8.6	-	91
61	413371	70	F	6	O	+	-	+	-	-	28.7	98	176	154	60	146	38	150	100	31.0	11.67	21.0	266	0.6	9.4	T	105
62	413539	67	M	6	O	+	+	+	-	+	30.8	106	144	198	90	151	40	140	90	42.0	20.33	26.0	360	0.9	8.4	-	89
63	413719	52	M	15	I	+	+	+	-	-	31.6	108	200	210	94	171	42	140	90	41.0	21.05	29.0	394	1.3	10	-	62
64	416501	62	F	12	O	+	-	+	-	-	28.9	94	136	180	68	156	54	140	90	32.0	14.08	28.0	246	1.0	8.9	2	60
65	415830	62	F	10	O	+	-	-	-	-	27.27	92	146	156	60	146	44	150	100	12.0	4.58	8.0	162	1.4	8.6	-	40
66	415898	70	M	20	I	+	+	+	-	+	29.39	101	136	210	86	190	34	150	90	36.0	18.48	21.0	296	1.2	11	4	64
67	415922	56	M	6	O	+	+	+	-	-	30.01	108	108	218	90	160	42	160	100	42.0	22.38	32.0	306	1.1	7.2	-	74
68	415936	62	M	12	O	+	-	-	-	+	28.01	100	136	210	86	142	46	140	90	22.0	11.29	11.0	190	0.6	8.9	-	145
69	415997	56	M	8	O	+	-	+	-	+	27.67	98	146	200	67	156	56	140	90	29.0	14.18	19.0	218	0.6	5.7	1	148
70	416006	58	M	9	O	+	-	+	-	-	30.8	110	142	210	76	160	60	150	100	34.0	17.45	20.0	258	0.7	8.8	2	123
71	416025	60	M	12	O	+	+	-	-	-	28.8	98	110	150	60	140	32	150	90	31.0	11.37	15.0	232	0.6	7.5	T	146
72	4161633	66	F	22	I	+	-	-	-	-	27.9	96	101	180	68	150	50	140	90	12.0	5.28	8.0	180	1.6	8.7	-	34
73	416164	71	M	31	I	+	+	+	-	+	32.3	105	136	190	70	169	42	160	100	39.0	18.11	32.0	318	0.7	12	2	118
74	416147	50	M	8	O	-	-	+	+	+	29.3	99	138	190	88	146	52	130	60	32.0	14.86	18.0	256	1.0	8	1	84
75	416221	65	M	9	O	+	-	-	-	-	31.6	110	120	160	56	160	52	160	100	26.0	10.17	10.0	230	2.0	8.4	T	36
76	416486	62	F	13	O	+	+	-	+	+	30.8	104	150	180	70	146	46	140	90	33.0	14.52	26.0	244	0.7	9.7	2	90
77	416491	52	F	4	O	+	+	+	-	+	31.9	118	146	220	90	168	44	150	100	46.0	24.74	33.0	361	0.7	8.3	T	93
78	416605	58	M	2	O	+	-	+	-	-	27.8	96	130	178	89	160	54	140	90	33.0	14.36	22.0	240	1.1	7.2	-	73
79	416639	48	M	3	O	+	-	+	-	-	27.8	96	110	180	68	178	30	160	100	32.0	14.08	26.0	262	1.4	8.2	-	57
80	416682	52	F	12	O	+	-	-	-	+	26.9	96	146	150	56	130	56	150	90	20.0	7.33	11.0	170	0.6	12	2	112
81	416795	54	M	7	O	-	+	+	-	-	29.8	103	160	190	88	170	32	120	80	40.0	18.58	30.0	288	0.6	6.8	-	149
82	416897	70	M	21	I	+	-	+	-	-	29.01	101	110	190	76	176	30	160	100	36.0	16.72	26.0	284	0.8	11	1	102
83	416947	60	F	12	O	+	-	-	-	-	26.6	94	110	160	58	142	46	150	100	18.0	7.04	8.0	174	0.9	6.9	T	68
84	417027	60	F	8	O	+	+	+	+	+	32.8	112	160	256	96	176	36	160	90	46.0	28.79	30.0	401	1.9	8.6	1	29
85	417128	80	M	27	I	+	+	+	-	-	29.8	102	150	190	88	146	42	150	100	34.0	15.79	38.0	346	5.0	14	4	12
86	417218	68	M	15	O	-	-	-	-	+	37.8	100	160	200	60	208	36	120	80	19.0	9.29	22.0	216	1.1	13	T	71
87	416510	62	F	11	O	+	-	+	-	-	29.6	102	110	180	96	156	48	150	100	30.0	13.20	28.0	248	0.4	8.8	2	172
88	417227	46	M	2	O	-	+	-	-	-	28.9	101	160	200	106	218	36	130	80	40.0	19.56	38.0	296	0.5	7.2	-	190
89	417481	76	F	21	I	+	+	+	-	+	32.3	106	176	190	110	188	39	140	90	45.0	20.90	39.0	356	0.7	12	3	86
90	416398	65	M	12	I	+	+	+	+	+	32.6	108	168	290	148	190	36	170	100	57.0	40.41	48.0	406	1.0	13	4	80
91	417577	56	M	6	O	-	-	-	-	-	30.6	102	118	280	108	220	36	120	80	22.0	15.06	8.0	190	1.1	6.9	1	74
92	416636	80	M	23	I	+	+	+	-	-	31.6	106	120	190	60	166	52	160	100	41.0	19.04	36.0	310	0.9	9.4	2	86
93	417600	66	M	12	O	-	+	-	+	+	30.9	100	156	200	89	140	36	120	80	34.0	16.62	26.0	258	3.6	11	3	18
94	416467	58	F	6	O	+	-	+	-	-	27.1	94	190	200	96	166	38	150	100	31.0	15.16	27.0	251	0.7	11	-	91
95	417690	54	M	4	O	+	-	+	-	-	37.8	110	122	178	110	160	50	160	100	36.0	15.66	32.0	310	0.9	9.4	1	93
96	416131	50	M	5	O	-	-	-	-	+	26.6	94	136	180	64	141	44	120	70	20.0	8.80	9.0	188	0.6	6.7	-	152
97	417361	77	M	12	O	+	+	+	-	-	30.8	100	112	200	98	198	36	180	100	44.0	21.51	38.0	318	1.3	9.7	1	57
98	417409	49	M	5	O	+	+	-	-	-	30	104	156	180	86	166	38	150	100	41.0	18.04	40.0	301	2.4	9.7	2	31
99	416418	78	M	22	I	+	+	+	-	+	32.8	110	176	240	121	180	41	100	100	52.0	30.51	40.0	410	0.9	12	2	87
100	417421	80	F	22	I	+	+	+	-	+	31	98	150	220	106	158	32	140	90	42.0	22.59	36.0	396	1.4	5.4	4	38

Annexures

Annexure III

ANNEXURE III – KEY TO MASTER CHART

BMI	– Body mass index
BP	– Blood pressure
Cholesterol	– Total Cholesterol
CVA	– Cerebrovascular accidents
CVD	– Cardiovascular disease
Dias	– Diastolic blood pressure
DR	– Diabetic retinopathy
eGFR	– Estimated glomerular filtration rate
FBS	– Fasting blood sugar
FIL	– Fasting insulin levels
HbA1c	– Glycated haemoglobin
HDL	– High density lipoproteins
HOMA-IR	– Homeostatic model assessment – Insulin resistance
hsCRP	–Highly sensitive C-reactive protein
HTN	– Hypertension
IL-18	– Interleukin 18
IP no.	– In-Patient number
LDL	– Low density Lipoprotein
PN	– Peripheral neuropathy
Sys	– Systolic blood pressure
T2DM	– Type 2 diabetes mellitus
TG	– Triglycerides
WC	– Waist circumference