"ASSOCIATION OF SERUM ADIPONECTIN LEVELS WITH CHRONIC KIDNEY DISEASE STAGE 2 TO STAGE 4 IN PATIENTS WITH TYPE 2 DIABETES MELLITUS - A ONE YEAR CROSS-SECTIONAL STUDY"

By

Dr. THACKER VINIT ARVINDBHAI
REG NO. BG0111010

Dissertation

Submitted to the
KLE University, Belgaum, Karnataka

In Partial Fulfillment
of the requirements for the degree of

M. D.
in
GENERAL MEDICINE

Under the Guidance of

Dr. MALLIKARJUN S. KHANPET **MD,DNB (Nephrology)**
Professor

DEPARTMENT OF MEDICINE,
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KARNATAKA

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I hereby declare that this dissertation entitled "**ASSOCIATION OF SERUM ADIPONECTIN LEVELS WITH CHRONIC KIDNEY DISEASE STAGE 2 TO STAGE 4 IN PATIENTS WITH TYPE 2 DIABETES MELLITUS - A ONE YEAR CROSS-SECTIONAL STUDY"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. MALLIKARJUN S. KHANPET** **MD, DNB (Nephrology)** Professor, Department of Medicine, Jawaharlal Nehru Medical College, Nehru Nagar, Belgaum – 590010.

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LIST OF ABBREVIATIONS USED

α	-	Alpha
β	-	Beta
µg/mL	-	Microgram per milli litre
ACCORD	-	Action to Control Cardiovascular Risk in Diabetes
ACE	-	Angiotensin-converting enzyme
Acrp30	-	Adipocyte complement-related protein of 30 kilodalton
AD	-	Anno Domini
AGEs	-	Advanced glycation end-products
apos	-	Apolipoproteins
ARBs	-	Angiotensin receptor blockers
ATP	-	Adenosine triphosphate
BC	-	Before Christ
BMI	-	Body mass index
cAd	-	Collagenous domain
CAD	-	Coronary artery disease
CDC	-	Centers for Disease Control
cDNA	-	Complementary deoxyribonucleic acid
CHF	-	Congestive heart failure
CKD	-	Chronic kidney disease
CVD	-	Cardiovascular disease
DCCT	-	Diabetes Control and Complications Trial
DKA	-	Diabetic ketoacidosis
DKD	-	Diabetic kidney disease
DM	-	Diabetes Mellitus

DNA	-	Deoxyribo nucleic acid
EDIC	-	Epidemiology of Diabetes Interventions and Complications
eGFR	-	Estimated glomerular filtration rate
ELISA	-	Enzyme linked immuno sorbant assay
ESRD	-	End stage renal disease
FFAs	-	Free fatty acids
FP	-	Foot process
FPG	-	Fasting plasma glucose
gAd Tg	-	Globular adiponectin transgenic
gAd	-	Globular domain
GBP28	-	Gelatin binding protein of 28 kilodalton
GDM	-	Gestational diabetes mellitus
GFR	-	Glomerular filtration rate
GIR	-	Glucose infusion rate
h	-	Hour
HbA1c	-	Glycated hemoglobin
HDL	-	High density lipoprotein
HHS	-	Hyperglycemic hyperosmolar state
HNF	-	Hepatocyte nuclear transcription factor
hs-CRP	-	High-sensitive C-reactive protein
i.e.	-	That is
ICMR	-	Indian Council of Medical Research
IDDM	-	Insulin dependent diabetes mellitus
IDF	-	International Diabetes Federation
IFG	-	Impaired fasting glucose

IGT	-	Impaired glucose tolerance
IPF	-	Insulin promoter factor
IRS	-	Insulin receptor substrate
KDOQI	-	Kidney Disease Outcomes Quality Initiative
Kg	-	Kilogram
lbs	-	Pounds
m	-	Meter
MDRD	-	Modification of Diet in Renal Disease
mg	-	Milligram
mg/dL	-	Milligram per decilitre
mL/min	-	Milli Liter per minute
mmol/L	-	Milli mole per liter
MODY	-	Maturity onset diabetes of young
n	-	Total number
NCEP	-	National Cholesterol Education Program
NF	-	Nuclear factor
NGT	-	Normal glucose tolerance
NIDDM	-	Non insulin dependent diabetes mellitus
NKF	-	National Kidney Foundation
NPH	-	Neutral Protamine Hagedorn
^{o}C	-	Degree centrigrade
p	-	Probability
PAD	-	Peripheral arterial disease
PAI-1	-	Plasminogen activator inhibitor type 1
PI-3K	-	Phosphatidylinositol 3-kinase

PKA	-	Protein kinase A
PODIS	-	Prevalence of Diabetes in India Study
PPAR	-	Peroxisome proliferator-activated receptors
PTX	-	Paclitaxel
RAS	-	Renin-angiotensin system
RNA	-	Ribonucleic acid
ROS	-	Reactive oxygen species
RR	-	Relative risk
SAA3	-	Serum amyloid A3
SD	-	Standard deviation
TNF	-	Tumor necrosis factor
TZD	-	Thiazolidinedione
UKPDS	-	United Kingdom Prospective Diabetes Study
VADT	-	Veterans Affairs Diabetes Trial
VEGF	-	Vascular endothelial growth factor
VLDL	-	Very Low Density Lipoprotein
WHO	-	World Health Organization
γ	-	Gamma

ABSTRACT

Background and objectives

Adiponectin plays an important role in the pathogenesis of type 2 DM. In kidney disease, higher adiponectin levels are present in dialysis patients but not in nondiabetic patients with predialysis chronic kidney disease. The present study was aimed to find the association of serum adiponectin levels with stage 2 to stage 4 of Chronic Kidney Disease in patients with type 2 Diabetes mellitus.

Methodology

The present one year cross sectional study was done at Department of Nephrology/Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. A total of 100 patients with type 2 diabetes mellitus and chronic kidney disease admitted during the study period from January 2012 to December 2012 were studied.

Results

In this study, maximum patients were in the age group of >60 years (53%) and the mean age was 64.4 ± 10.23 years. Of the 100 patients, 61% were males and 39% were females with male to female ratio of 1.56 to 1. The duration of diabetes was 6 to 10 years in 43% of the patients. Maximum number of patients were in the stage of II to III chronic kidney disease (86%) and 14% were in stage IV chronic kidney disease. In 26% of the patients, the adiponectin levels were below normal and in 5% they were more than normal.

Conclusion and interpretation

Advanced stage of the CKD in patients with T2DM was associated with low levels of adiponectin as higher number of patients with stage IV CKD (42.86%) had serum adiponectin levels ≤ 8.3 μg/mL. Low levels of serum adiponectin were also associated with age, gender, creatinine and proteinuria.

Keywords

Adiponectin; Diabetic kidney disease; Diabetes mellitus;

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Chapter 1

Introduction

INTRODUCTION

Diabetes Mellitus (DM) 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 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 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]

Rates of diabetes are increasing worldwide. Centers for Disease Control and Prevention (CDC) report in 2011 estimated that nearly 26 million Americans have diabetes.[3] 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.

The disease burden of DM is primarily due to the burden of its many complications. Diabetes exposure, which results from the level as well as duration of hyperglycemia, represents a metabolic state that favours the development of several long term complications of eyes, kidneys and heart.[4,5,6]

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 disease (CKD). Dyslipidemia and inflammation may promote renal disease via mechanisms of vascular endothelial cell dysfunction in type 2 diabetes mellitus (DM).[10]

Chronic kidney disease (CKD) is either decreased glomerular filtration rate (GFR) or albuminuria, or both, carries a risk of cardiovascular morbidity and mortality and progression to end-stage renal disease.[11-13] It is a worldwide public

health problem affecting more than 50 million people, and more than one million of them are receiving kidney replacement therapy. As the population of patients with diabetes of long duration grows, reports of a dramatically increasing burden of diabetic kidney disease (DKD) are appearing from developed countries, as well as from Africa, India, the Pacific Islands, and Asia, where infectious disease previously posed the greatest threat. Increased risk and more rapid progression of DKD also have been reported in immigrants from developing to developed countries.[14]

Systemic inflammation has been implicated in the progression of chronic kidney disease in animal models and in humans. As the leading cause of kidney failure in the world, type 2 diabetes has been postulated to be a generalized inflammatory condition resulting from obesity-induced dysregulation of adipocytes, which produce an excess of inflammatory cytokines. Scientists have speculated that this persistent inflammatory state further contributes to the development of the extensive vascular disease characteristic of diabetes.[15]

Adiponectin, a recently discovered circulating 30-kDa protein exclusively secreted by adipocytes, is present at concentrations of 5–30 μg/ml in healthy humans[16] and is considered to be an important modulator of insulin sensitivity and dyslipidemia.[18] Anti-inflammatory properties also have been attributed to adiponectin, a theory supported by observations that serum concentrations of adiponectin are inversely associated with inflammatory markers such as fibrinogen, intracellular adhesion molecule-1, E-selectin, and C-reactive protein.[15] The observation that adiponectin may be protective against vascular disease via the above mechanisms is supported by cross-sectional analyses of

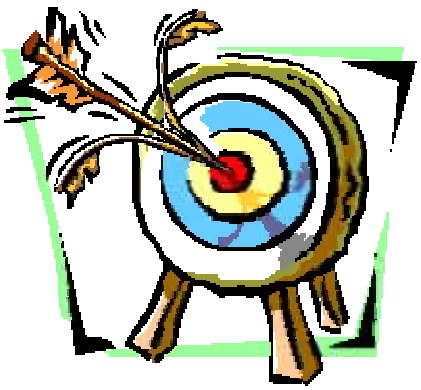
individuals with coronary heart disease, who have lower concentrations of adiponectin when compared with control subjects,[19,20] and prospective studies revealing that higher adiponectin is associated with a decreased risk for subsequent cardiovascular disease events in nondiabetic subjects,[21] type 1 diabetic subjects,[22] type 2 diabetic men[23] and in end-stage renal disease patients.[24] The role of adiponectin in cardiovascular disease is not definitive, however, because some studies have found no relation between adiponectin and cardiovascular disease risk.[25,26]

Adiponectin appears to play an important role in the pathogenesis of type 2 diabetes. Cross-sectional studies[27-30] have demonstrated that serum concentrations of adiponectin are decreased in type 2 diabetic subjects compared with nondiabetic control subjects. Moreover, higher adiponectin levels are associated with better lipid and glycemic control in type 2 diabetic subjects. One prospective study[31] has reported that lower baseline serum adiponectin appears to be a harbinger for the development of type 2 diabetes. In kidney disease, higher adiponectin levels are present in dialysis patients but not in nondiabetic patients with predialysis chronic kidney disease when compared with healthy control subjects.[15]

However, to date the association of adiponectin with kidney function in individuals with type 2 diabetes is not well described. The existing literature has focused mainly on those with glucose intolerance or end-stage renal disease. Hence the present study was to find the association of serum adiponectin levels with stage 2 to stage 4 of Chronic Kidney Disease in patients with type 2 Diabetes mellitus.

Chapter 2

Objectives



OBJECTIVES

The objective of the study was to find the association of serum adiponectin levels with stage 2 to stage 4 of Chronic Kidney Disease in patients with type 2 Diabetes mellitus.

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.[6,32]

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.[32]

- 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.[32]

- 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.[32]

- 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.[32]

- In 1889, Oskar Minkowski and Josef Von Mering observed that total pancreatectomy produced diabetes in dogs.[32]

- 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.[32]

- 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.[32]

- 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.[32]

- 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
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.[1,33]

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.[1,6,33-35]

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[36]

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.[37]

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.[38] 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.[38]

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.[39]

Sex

Type 2 DM is slightly more common in older women than men.[39]

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.[39]

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.[6,33]

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.[6,33]

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.[37] 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.[40] 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.[37]

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).[6,33]

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.[33]

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.[41]

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]

Table No. 1: Classification of weight status and risk of disease[42]

	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[32,33]

- 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]

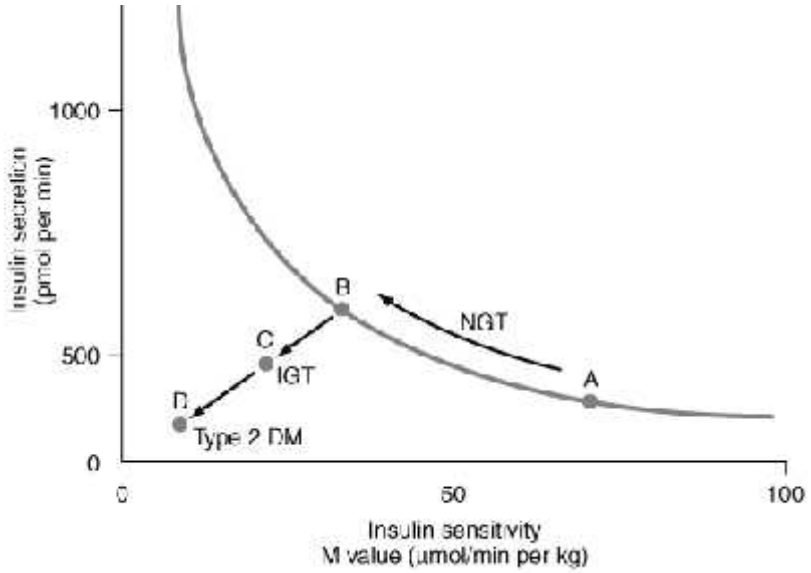


Figure 1. Metabolic changes during the development of type 2 diabetes mellitus[1]

COMPLICATIONS OF DIABETES MELLITUS[6,33]

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.[6,33]

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.[33]

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.[35]

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.[43,44]

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.[45,46]

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.[6,46-48]

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.[45-47]

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.[46]

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.[49-50]

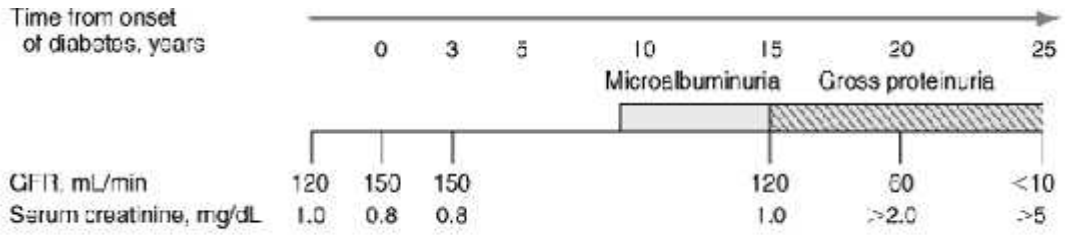


Figure 2. Time course of development of diabetic nephropathy

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.[49]

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 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 kidney disease.[48,51]

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.[52,53]

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.[54]

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.[52,54,55]

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.[51,56]

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.[33,51]

In overt diabetic nephropathy an overall decrease of glomerular filtration rate is present at the same time with proportional enhancement of albumin filtering. However, 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.[55,57]

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.[54]

Related to albuminuria, the results of previous study favor the glomerular filtration rate estimation to define kidney disease stage in diabete mellitus patients.[58]

Chronic kidney disease in type 2 diabetes mellitus

The Kidney Disease Outcomes Quality Initiative (K/DOQI) of the National Kidney Foundation (NKF) defines chronic kidney disease as either kidney damage or a decreased glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m^2 for 3 or more months.[14]

Diabetes mellitus (DM) now accounts for more cases of end-stage renal disease (ESRD) than any other cause of chronic kidney disease (CKD). In North America, four out of every 10 new cases of ESRD arise due to diabetic kidney disease (DKD), a proportion that has risen steadily over the past few decades and shows no signs of slowing. Roughly 30% of individuals with type 1 and 10% of those with type 2 diabetes will develop DKD. Given the epidemic levels of obesity and type 2 DM, a comprehensive understanding of the etiology of DKD is urgently needed.[59]

Hyperglycemia, the defining feature of diabetes, is a fundamental cause of vascular target organ complications, including diabetic kidney disease (DKD). Intensive treatment of hyperglycemia prevents elevated albuminuria or delays its progression, but patients treated by approaches designed to achieve near normal glycemia may be at increased risk of severe hypoglycemia. Evidence that intensive treatment has an effect on loss of glomerular filtratin rate (GFR) is sparse.[60]

The evidence that achieving an HbA1c level of ≤ 7.0% is able to prevent the microvascular complications of diabetes was presented in detail in the original KDOQI diabetes guideline.[4] For type 1 diabetes, evidence from the

Diabetes Control and Complications Trial (DCCT),[61,62] as well as from a metaanalysis of a number of smaller studies that preceded the DCCT,[63] established that this level of glycemic control decreases the risk of microalbuminuria and retinopathy compared to less stringent control. The beneficial effects of intensive therapy on these outcomes persisted during the long-term follow-up study of the DCCT subjects, called the Epidemiology of Diabetes Interventions and Complications (EDIC) Study. Despite the gradual narrowing of the difference in HbA1c levels between the two DCCT groups over the first two years in the follow-up period, and levels remaining near 8% for both groups for the subsequent 12 years, the reduction in risk of microvascular complications of diabetes persisted.[64] Similar benefits of glycemic control on the development of microalbuminuria in patients with type 2 diabetes were originally observed in three studies; the Kumamoto Study,[65,66] the United Kingdom Prospective Diabetes Study (UKPDS),[67,68] and the Veterans Affairs Cooperative Study on Glycemic Control and Complications in Type 2 Diabetes Feasibility Trial.[69] Intensive glycemic control also significantly reduced the development of macroalbuminuria in patients with type 1 diabetes, as shown in the DCCT/EDIC Study[61-63] as well as the similarly designed but smaller Stockholm study,[70] and in those with type 2 diabetes, as shown in the Kumamoto study[65,66] and the VA Cooperative Study.[69] The UKPDS showed a trend toward decreased development of macroalbuminuria, but this result did not achieve statistical significance.[67,68]

Three new studies have added to the evidence that even more intensive glycemic control reduces the development of elevated albuminuria in patients with type 2 diabetes. In the Action in Diabetes and Vascular Disease: Preterax

and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial,[71] more intensive control that achieved an HbA1c of 6.5%, compared with standard control (HbA1c 7.3%), was associated with a 21% reduction in new onset or worsening nephropathy defined by new onset macroalbuminuria, doubling of Serum Creatinine, need for kidney replacement therapy, or death due to kidney disease (4.1% vs. 5.2%) Additionally, intensive glycemic control reduced development of macroalbuminuria by 30% (2.9% vs. 4.1%), and development of new onset microalbuminuria by 9% (23.7% vs. 25.7%).[71]

The Action to Control Cardiovascular Risk in Diabetes (ACCORD) study similarly showed that more intensive control, achieving an HbA1c of 6.4%, compared with standard control (HbA1c 7.6%), resulted in a 32% reduction in the development of incident macroalbuminuria (2.7% vs. 3.9%) and a 21% reduction in the development of incident microalbuminuria (12.5% vs. 15.3%).[72]

In the Veterans Affairs Diabetes Trial (VADT), more intensive glycemic control that achieved an HbA1c of 6.9% compared with standard control (HbA1c 8.4%) resulted in a 37% reduction in macroalbuminuria (7.6% vs.12.1%) and a 32% reduction in microalbuminuria (10.0% vs.14.7%).[73]

A few long-term observational cohort studies and secondary or post hoc analyses of interventional studies using ACE-Is or ARBs found that poorer glycemic control is associated with a greater rate of fall of GFR in patients with type 1 diabetes.[74-78] Most of the prospective, randomized studies used as evidence for the effect of glycemic control on kidney function are limited by the small numbers of patients reaching this intermediate outcome. However, the

EDIC/DCCT follow-up study recently reported that 2.0% (1.6/1000 person-years) of participants in the previously intensive treatment group and 5.5% (3.0/1000 person years) of those in the previously conventional treatment group developed sustained estimated glomerular filtration rate (eGFR) measurements < 60 mL/min/1.73 m2 with a relative risk (RR) reduction of 50% (p=0.006); there were similar RR reductions for single eGFR measurements < 45 mL/min/1.73 m2 (50%, 1.6/1000 person-years vs. 2.5/1000 person-years, p=0.045) and < 30 mL/min/1.73 m2 (44%, 0.8/1000 person-years vs. 1.5/1000 person-years, p=0.088) and for ESRD (51%, 0.5/1000 person-years vs. 1.1/1000 person-years, p=0.098).[79]

For patients with type 2 diabetes, intensive treatment in the UKPDS was associated with a 67% risk reduction for a doubling of plasma creatinine levels at 9 years (0.71% of the intensive group and 1.76% of the conventional group, p=0.027).[67] None of the three more recent studies mentioned above (ADVANCE, ACCORD, VADT) showed significant benefits of more intensive glycemic control on creatinine-based estimates of GFR.[71-73]

Accordingly, the evidence that intensive glycemic control reduces the microvascular complications of diabetes is based almost exclusively on prevention of microalbuminuria (a predictor of actual complications), reduced progression to macroalbuminuria, and on prevention of retinopathy. Evidence for the prevention of other intermediate microvascular outcomes, including declining eGFR and doubling of Serum Creatinine, is sparse. Although there is no evidence that intensive glycemic control slows progression to the clinical endpoint of ESRD, it is likely that if the earlier manifestations of kidney disease are reduced

(i.e., albuminuria and earlier-stage CKD), then the eventual outcome of ESRD will also be reduced. However, such assumption presumes that benefits of intensive glycemic control are not outweighed by harms and that patients survive to reach ESRD.[60]

KDOQI practice guidelines recommend not treating to an HbA1c target of <7.0% in patients at risk of hypoglycemia.[60]

The major risk of attaining HbA1c levels <7.0% in people with diabetes is hypoglycemia. Risk of hypoglycemia is amplified in those with CKD, especially if kidney function is substantially reduced (CKD stages 4 and 5). At HbA1c levels <7.0%, increased risk of hypoglycemia is clearly evident for patients with type 1 diabetes.[61,70,80] Although the Kumamoto Study and UKDPS also demonstrated an increased risk of hypoglycemia in those with type 2 diabetes treated with insulin,[65,66] the magnitude of the risk was considerably less than in type 1 diabetes. The UKPDS also showed that sulfonylureas are associated with a small risk of hypoglycemia.[67] The three most recent clinical trials (ADVANCE,ACCORD, and VADT) all showed substantial increases (range 1.5-3 fold) in severe and non-severe hypoglycemia among patients with type 2 diabetes who were receiving more intensive therapy.

Intensifying glycemic control beyond conventional management did not result in decreased risk of the primary endpoints, defined by composites of major adverse cardiovascular disease (CVD) events, in any of these studies.[71,73] Moreover, there was an increase in all cause mortality among the intensively-treated group compared to the conventionally-treated group in the ACCORD

study.[81] The reasons for this finding are uncertain, although further analysis showed that increased mortality was not directly attributable to hypoglycemia.[82] Therefore, lowering HbA1c to levels <7.0% is not recommended in patients with diabetes who are at risk for hypoglycemia, including those treated with insulin or sulfonylureas and/or have advanced CKD.[60]

KDOQI practice guidelines suggest that target HbA1c be extended above 7.0% in individuals with co-morbidities or limited life expectancy and risk of hypoglycemia.[60]

Risks of microvascular complications are amplified with progressively increasing levels of HbA1c. Good glycemic control is clearly fundamental to optimal diabetes care. However, the available evidence is insufficient to specify an upper limit for target HbA1c. Nevertheless, the ADVANCE, ACCORD, and VADT studies can provide some insight into setting goals for individual patients. For example, study participants (characteristically older people with long-standing type 2 diabetes and high frequency of CVD and other co-morbidities) treated in the conventional manner were less likely to experience hypoglycemia, while risks of major clinical endpoints (all-cause mortality, CVD mortality, non-fatal CVD events, and loss of kidney function or ESRD) were similar to those treated more intensively. The achieved HbA1c values among the conventional treatment groups in these studies were 7.3-8.4%.[60]

Years of intensive glycemic control (HbA1c ≈ 7%) are required before a reduction in the incidence of complications, such as kidney failure or blindness, becomes evident. Therefore, when instituting intensive therapy for

hyperglycemia in patients with limited life expectancy, the potential benefits must be balanced against risks. With intensified insulin treatment, there is an increased risk of hypoglycemia and weight gain. In individuals 70-79 years of age who are taking insulin, the probability of falls begins to increase with HbA1c <7%. Moreover, in patients with type 2 diabetes, one study showed that the presence of co-morbidities abrogates benefits of lower HbA1c levels on CVD events. Therefore, a target HbA1c of ≈7.0% is suggested for patients with diabetes who are at risk of hypoglycemia and have clinically-significant co-morbidities or limited life expectancy.[60]

The risk of hypoglycemia is increased in patients with substantial decreases in eGFR (CKD stages 4 and 5) for two reasons: (1) decreased clearance of insulin and of some of the oral agents used to treat diabetes and (2) impaired renal gluconeogenesis with reduced kidney mass. The contribution of reduced renal function to the risk of hypoglycemia is difficult to quantify. About one-third of insulin degradation is carried out by the kidneys and impairment of kidney function is associated with a prolonged half-life of insulin. Patients with type 1 diabetes receiving insulin who have significant creatinine elevations (mean 2.2 mg/dL) have a 5-fold increase in the frequency of severe hypoglycemia.[60]

Microalbuminuria is an early clinical marker of DKD that results from damage to the glomerular filtration barrier at the level of the highly differentiated glomerular podocyte cells. Injury to these epithelial cells, podocytopathies, includes cellular hypertrophy, foot process effacement, detachment from the glomerular basement membrane, and apoptosis.[60]

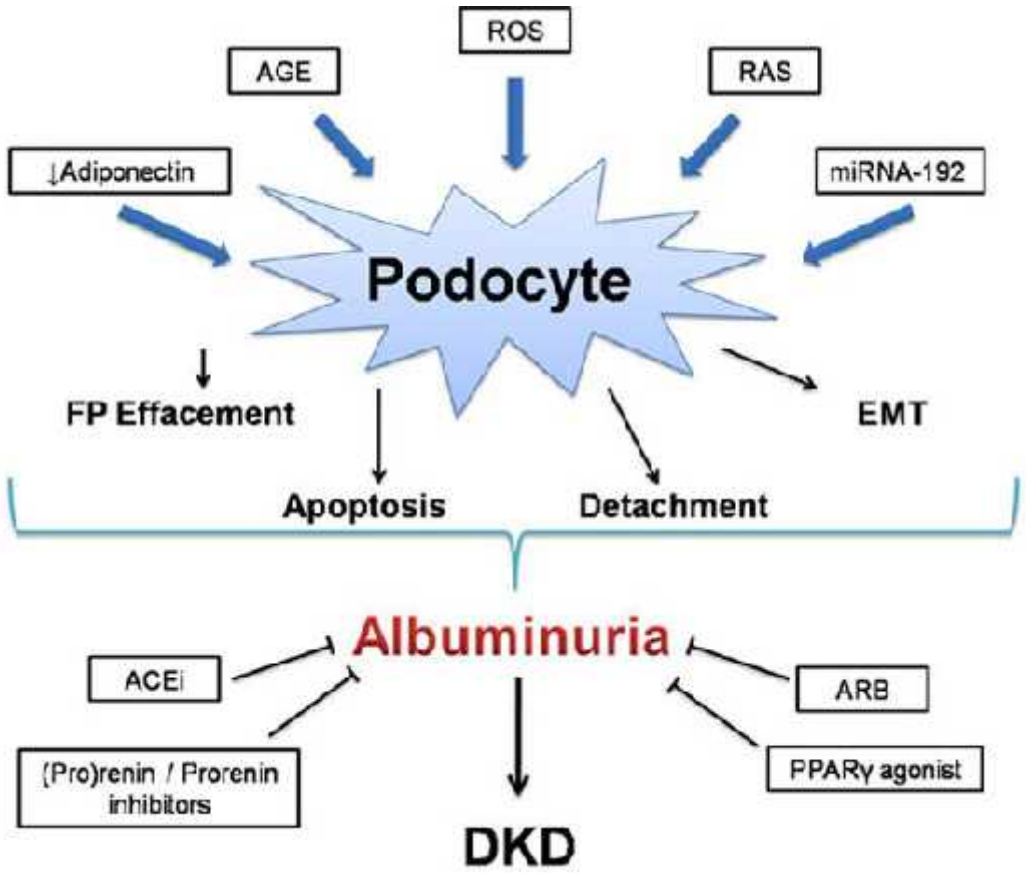


Figure 3. Mediators of podocyte injury in DKD. Several molecular pathways are known to contribute to podocyte injury in DKD. As depicted, these include, but are not limited to, the following: decreased adiponectin, production of advanced glycation end-products (AGEs), increased reactive oxygen species (ROS), activation of the RAS, and miRNA-192 all contribute to podocyte injury. Podocyte injury exhibits itself as foot process (FP) effacement, apoptosis, detachment, and EMT. Damage to the podocyte leads to increased albuminuria and exacerbates DKD. Agents known to inhibit this process include ARBs, ACEi, peroxisome proliferator-activated receptors (PPAR) agonists, and (pro)renin/prorenin inhibitors.[59]

The role of a number of recently identified factors that contribute to podocytopathies in DKD include members of the renin-angiotensin system (RAS), including angiotensin-converting enzyme (ACE) types 1 and 2, prorenin and its receptor, reactive oxygen species (ROS), prostanoids, peroxisome

proliferator-activated receptors (PPAR), advanced glycation end-products (AGEs) and their receptors (RAGE), adiponectin, and microRNAs. As the number of therapeutic options that slow, but do not halt, the progression of DKD to ESRD remains limited, a more comprehensive understanding of the signaling events that contribute to this increasingly prevalent disease may identify novel avenues for treatment and prevention.[59]

Adiponectin

Produced in adipose tissue, adiponectin is an important peptide hormone involved with glucose regulation and fatty acid catabolism. Decreased adiponectin levels result in oxidative stress, fusion of podocyte foot processes, and microalbuminuria.[83-85] Consistent with these findings, adiponectin knockout mice have increased susceptibility to podocyte injury in a subtotal renal ablation model of progressive CKD.[86] Based on such studies, some have suggested that adiponectin may be a biomarker for kidney disease and, due to its involvement in protecting the filtration barrier, a useful therapeutic target in slowing DKD progression.[87] Evidence to support a role for adiponectin in podocyte function is accumulating. A recent study by Cammisotto and Bendayan revealed that stimulation of the adiponectin receptor in podocytes yielded activation of AMP-activated protein kinase, which controls oxidative stress and apoptosis.[88]

The adipocyte and adipose tissue

Adipose tissue is a hormonally active tissue, producing adipocytokines which may influence activity of other tissues. Adiponectin, abundantly present in the plasma increases insulin sensitivity by stimulating fatty acid oxidation,

decreases plasma triglycerides and improves glucose metabolism. Adiponectin levels are inversely related to the degree of adiposity. Anorexia nervosa and type 1 diabetes are associated with increased plasma adiponectin levels and higher insulin sensitivity. Decreased plasma adiponectin levels were reported in insulin-resistant states, such as obesity and type 2 diabetes and in patients with coronary artery disease. Activity of adiponectin is associated with leptin, resistin and with steroid and thyroid hormones, glucocorticoids, NO and others. Adiponectin suppresses expression of extracellular matrix adhesive proteins in endothelial cells and atherosclerosis potentiating cytokines. Anti-atherogenic and anti-inflammatory properties of adiponectin and the ability to stimulate insulin sensitivity have made adiponectin an important object for physiological and pathophysiological studies with the aim of potential therapeutic applications.[89]

Adipose tissue is composed of the lipid-storing adipose cell and a stromal/vascular compartment in which preadipocytes reside. Adipose mass increases by enlargement of adipose cells through lipid deposition, as well as by an increase in the number of adipocytes. The process by which adipose cells are derived from a mesenchymal preadipocyte involves an orchestrated series of differentiation steps mediated by a cascade of specific transcription factors. One of the key transcription factors is peroxisome proliferator-activated receptor γ (PPARγ), a nuclear receptor that binds the thiazolidinedione class of insulin-sensitizing drugs used in the treatment of type 2 diabetes.

Recent research has shown that adipose tissue is not simply an inert storage depot for lipids but is also an important endocrine organ that plays a key role in the integration of endocrine, metabolic, and inflammatory signals for the

control of energy homeostasis. The adipocyte has been shown to secrete a variety of bioactive proteins into the circulation. These secretory proteins, which have been collectively named adipocytokines,[90] include leptin,[91] tumor necrosis factor (TNF)-alpha,[92] plasminogen activator inhibitor type 1 (PAI-1),[93] adipsin,[94] resistin,[95] and adiponectin.[96] Adiponectin, the gene product of the adipose most abundant gene transcript one (apM1),[96] is a novel and important member of the adipocytokine family.

Synthesis of Adiponectin

Adiponectin cDNA was first isolated by large scale random sequencing of the human adipose tissue cDNA library.[96] It is a collagen-like protein that is exclusively synthesized in white adipose tissue, is induced during adipocyte differentiation, and circulates at relatively high (microgram/milliliter) concentrations in the serum. Both murine and human forms of adiponectin have been isolated independently by several groups, and various descriptive names have been given to the same compound by different investigators: adipocyte complement-related protein of 30 kilodalton (Acrp30),[97] Adipo Q[98] and gelatin binding protein of 28 kilodalton (GBP28).[99] The former two are murine analogs and the latter the human counterpart.

Properties of Adiponectin

Adiponectin has been postulated to play an important role in the modulation of glucose and lipid metabolism in insulin sensitive tissues in both humans and animals. Decreased circulating adiponectin levels have been demonstrated in genetic and diet-induced murine models of obesity,[100] as well as

in diet induced forms of human obesity.[101] Low adiponectin levels have also been strongly implicated in the development of insulin resistance in mouse models of both obesity and lipoatrophy.[100] In humans, plasma levels of adiponectin are significantly lower in insulin-resistant states including type 2 diabetes[102] and can be increased upon administration of the insulin-sensitizing thiazolidinedione (TZD) class of compounds.[103] Plasma adiponectin levels in diabetic subjects with coronary artery disease (CAD) are lower than in diabetic patients without CAD, suggesting that adiponectin may have anti-atherogenic properties.[104] In studies done on human aortic endothelial cells, adiponectin has been shown to dose-dependently decrease the surface expression of vascular adhesion molecules known to modulate endothelial inflammatory responses.[105] It also inhibits proliferation of vascular smooth muscle cells.[106] and concentrates within the vascular intima of catheter-injured vessels.[107] In clinical studies, low adiponectin levels have been associated with an atherogenic lipid profile.[104,108] The association of low adiponectin levels with obesity, insulin resistance, CAD, and dyslipidemia indicates that this novel protein may be an important new marker of the metabolic syndrome.

Structure and processing of Adiponectin

A description of the cDNA encoding adiponectin was first reported in 1995.[97] Adiponectin is a protein of 247 amino acids consisting of four domains, an amino-terminal signal sequence, a variable region, a collagenous domain (cAd), and a carboxy-terminal globular domain (gAd).[97] On the basis of both its primary amino acid sequence and its subunit domain structure, adiponectin is most similar to C1q, a member of the complement-related family of proteins.

However, X-ray crystallography of the globular fragment of adiponectin also reveals a striking structural homology to TNF-alpha, suggesting an evolutionary link between the TNF-alpha family members and adiponectin.[109] Once synthesized, mammalian adiponectin undergoes posttranslational hydroxylation and glycosylation yielding eight isoforms.[110] Six of the adiponectin isoforms are glycosylated. O-linked glycosylation sites have been mapped to four lysine residues, 68, 71, 80, and 104, and one proline residue, 94, located within the collagenous domain.[110]

Structure of Monomeric adiponectin

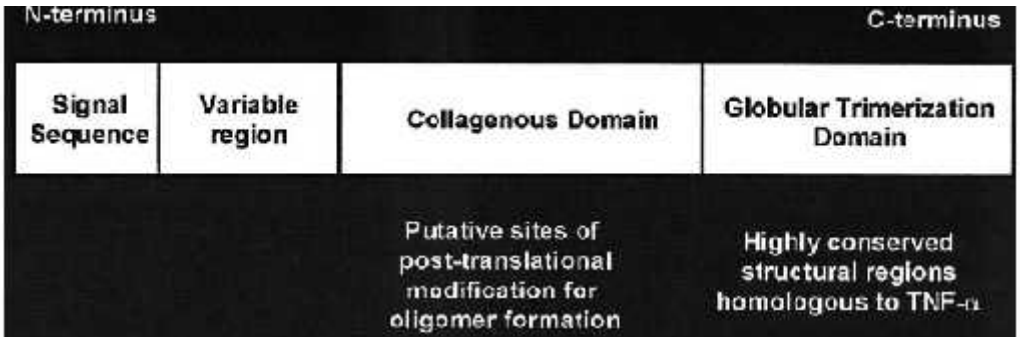


Figure 4. The domain structure of Acrp30: Signal sequence, species-specific variable region, collagenous domain and globular trimeization domain.

In addition, there is evidence that some of the O-linked glycans contain unique and adipocyte-specific disialic acid residues, a newly recognized class of sialyl groups in glycoproteins.[111] Functional analysis of full-length glycosylated mammalian adiponectin has revealed that it is significantly more potent as an insulin sensitizer than the recombinant nonglycosylated bacterial product. These observations suggest that posttranslational modifications of adiponectin may be necessary for optimal biological activity. The basic building block of adiponectin

is a tightly associated trimer, which is formed by association between three monomers at the globular domains. Monomeric (30-kDa) adiponectin has not been observed in the circulation and appears to be confined to the adipocyte. Four to six trimers associate through their collagenous domains to form higherorder structures, or oligomers, which circulate in plasma at concentrations of 5 to 30 g/ml.[97,101,109]

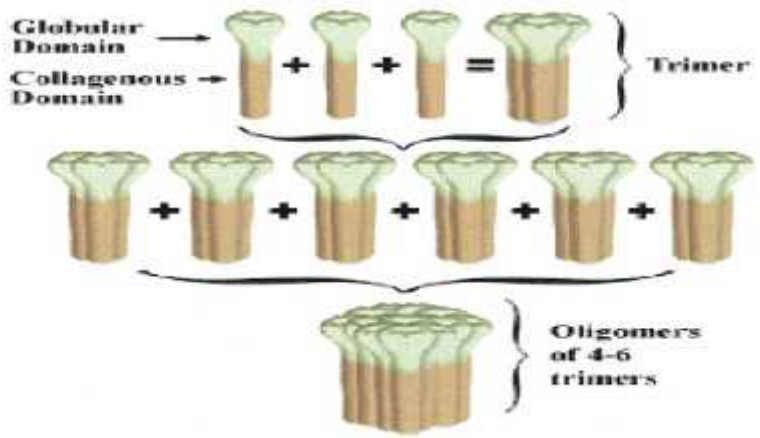


Figure 5. Model for assembly of adiponectin complexes. Three monomers form a trimer through associations between their globular domains. Four to six trimers associate noncovalently through their collagenous domains to form high–molecular-weight oligomers, which circulate in the plasma.

Without the collagenous domain, the globular domain of adiponectin still trimerizes but does not associate into higher-order structures.[109] Although the precise molecular mechanisms underlying the tight association of adiponectin trimers are not known, it is likely that interactions involving both the globular and the collagenous domains are important for ensuring the stability and activity of the multimeric forms.

Measurement of Adiponectin

The current methods available for measuring adiponectin in plasma include a radioimmunoassay (Linco, St Charles, MO) that measures the multimeric form and an enzyme-linked immunosorbent assay (B-Bridge International, San Jose, CA) that recognizes the denatured monomer form. Circulating levels detected with either method appear to be similar.

The pharmacological effects of adiponectin

The pharmacological effects have been studied at animal, tissue, and cellular levels using a variety of recombinant adiponectin products. Studies investigating the bioactivity of full length adiponectin versus that of the globular domain alone have produced mixed results. The globular head domain of adiponectin has been shown to be more potent than the full-length form in ameliorating hyperglycemia and hyperinsulinemia in diet-induced and genetic forms of murine obesity[11] and in decreasing elevated plasma free fatty acids in mice fed a high-fat meal or given intravenous intralipid injections.[112] These results are in contrast to those of other studies,[113] whereby injection of bacterially produced globular adiponectin into mouse models of type 1 and 2 diabetes did not induce a decrease in serum glucose, although the full-length form did. It is possible that adiponectin exists as variable protein complexes that exert different effects in various tissues.

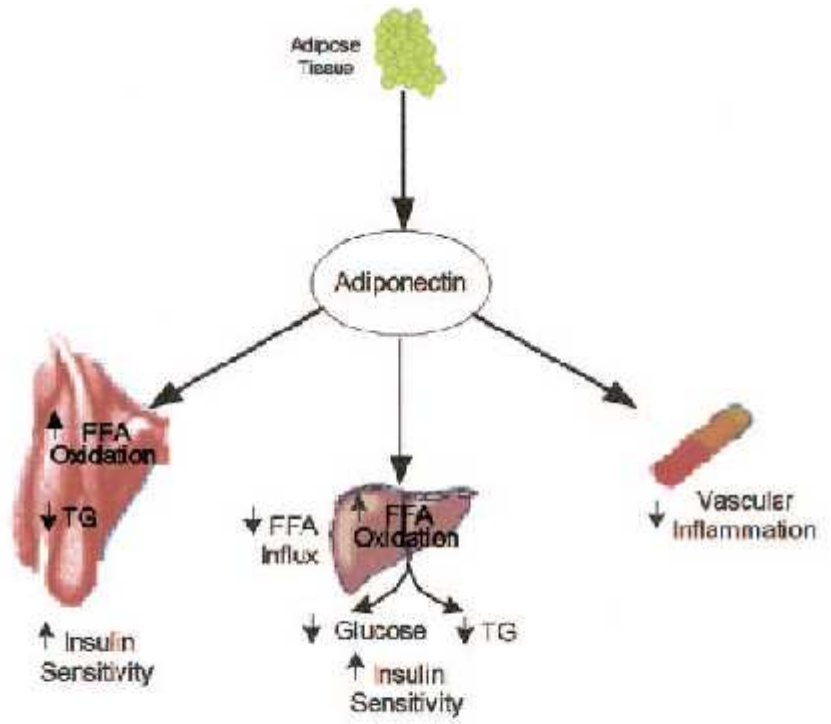


Figure 6. Hypothetical model for the actions of adiponectin.

In skelectal muscle, adiponectin increases tyrosine phosphorylation of the insulin receptor. This effect may contribute to increased insulin sensitivity. It also increases fatty acid oxidation, probably by activation 5'-AMP kinase, with resultant decreased intramyocellular steatosis. In the liver, the decreased free fatty acid influx and increased fatty acid oxidation contribute to reduced hepatic glucose outpur and VLDL (Very Low Density Lipoprotein) triglyceride synthesis. In vascular endothelium, adiponectin decreased monocyte adhesion to endothelium, adiponectin decreases monocyte adhesion to endothelium, suppresses macrophage to foam cell transformation and inhibits vascular smooth muscle cell proliferation and migration.

The mechanisms of action of adiponectin

The mechanisms of action are largely unknown and controversial. Adiponectin administration to rodents has been shown to increase insulin-induced tyrosine phosphorylation of the insulin receptor in skeletal muscle in association with increased whole-body insulin sensitivity.[100] These results were also validated in a recent study conducted in humans.[114] Stimulation of glucose utilization and fatty acid oxidation in skeletal muscle and liver by adiponectin may also occur through activation of 5-AMP kinase. 5-AMP–activated protein kinase is believed to play a crucial role in the regulation of energy expenditure and glucose and lipid metabolism. The tissue-specific effect of adiponectin on 5-AMP kinase has recently been demonstrated in mice. In these studies, both the globular and fulllength forms of adiponectin activated 5-AMP kinase in skeletal muscle, but only the full-length form stimulated phosphorylation and activation of AMP kinase in the liver.[115] In skeletal muscle of mice, adiponectin has been shown to increase expression of the genes encoding proteins involved in fatty acid transport and oxidation, such as CD36, acyl-CoA oxidase, and uncoupling protein, resulting in enhanced fat combustion and energy dissipation.[100] In the liver, low doses of adiponectin decreased the expression of proteins involved in fatty acid transport, such as CD36, leading to reduced fatty acid influx into the liver and hepatic triglyceride content.[100] Improved hepatic insulin sensitivity occurred, leading the investigators to postulate that the primary effects of adiponectin on muscle are to augment uptake and combustion of free fatty acids (FFAs), whereas decreased liver triglyceride content results from secondary reductions in serum FFA and triglyceride levels. In a separate experiment by the

same group,[116] amelioration of insulin resistance cell degranulation, and diabetes occurred in globular adiponectin transgenic (gAd Tg) crossed with leptindeficient ob/ob mice. Again, these findings were associated with increased skeletal muscle fatty acid oxidation. This finding is in contrast to that reported by another group in which, in the basal state, adiponectin exerted an insulin-sensitizing effect on hepatocytes with suppression of hepatic glucose output without a sustained attenuation of triglyceride accumulation in this tissue.[113] A unified theme for the method and site of adiponectin action thus remains to be determined.

Factors influencing Adiponectin levels

Although adiponectin is secreted only from adipose tissue, its levels are paradoxically lower in obese than in lean humans.[101] This is in contrast to most other adipocytokines, whose levels are increased in obesity in proportion to an increased total body fat mass. It is possible that although adiponectin expression is activated during adipogenesis, a feedback inhibition on its production may occur during the development of obesity. For example, adipocyte expression and secretion of adiponectin has been shown to be reduced by TNF- alpha.[117] Therefore it may be reasonable to surmise that increased TNF- alpha and possibly other adipocytokines that are expressed in increased amounts in the obese state may at least be partially responsible for the decreased adiponectin production in obesity. Levels are also lower in diabetic patients compared with nondiabetic subjects,[104] and are particularly low in subjects with CAD.[104] Decreased levels are found in men compared with women,[101] and this may be androgen induced.[118] The incidence of cardiovascular death has been found to be higher in patients with renal failure who have decreased adiponectin levels

(hypoadiponectinemia).[119] Decreased adiponectin levels were found to be closely related to the degree of insulin resistance and hyperinsulinemia in a study conducted on Pima Indians and Caucasians individuals with a wide range of glucose tolerance.[102] Several studies have reported a significant negative correlation between circulating adiponectin and triglyceride levels and a positive correlation between adiponectin and HDL cholesterol levels in type 2 diabetes.[104] Others have also demonstrated that plasma adiponectin concentrations were not only inversely related to triglyceride levels, atherogenic index (total: HDL cholesterol) and apolipoproteins (apos) B and E, but also positively correlated to serum HDL cholesterol and apo A-1 in nondiabetic female patients.[108] These declines in adiponectin in hypertriglyceridemic, high atherogenic index, and low HDL states were also observed after adjusting for BMI, body fat mass, age, and diastolic blood pressure. These findings suggest that the hypoadiponectinemia observed in dyslipidemia may accelerate the atherosclerotic changes seen in the metabolic syndrome.

Metabolic roles of adiponectin

Adiponectin as a mediator of insulin action/resistance

A strong correlation between adiponectin and systemic insulin sensitivity has been well established both in vivo and in vitro in mice, other animals, and humans.[100,113] In experiments conducted by researchers,[113] intraperitoneal injection of mammalian- expressed full-length adiponectin into fasting male wild-type mice and two models of type 1 diabetes insulinopenic nonobese diabetic and streptozotocin induced diabetic mice produced a significant transient reduction of

glucose levels. Adiponectin did not appear to be acting primarily as an insulin secretagogue, since insulin levels were low at the beginning of the experiments in all animals and remained low even after adiponectin injection. Adiponectin injection into a type 2 diabetic model (ob/ob mice) also lowered glucose levels.

Despite dramatically different insulin levels in the different mouse models, a common mechanism appeared to be responsible for the decreased plasma glucose sensitization of the liver to insulin-induced suppression of hepatic glucose output. Studies[100] showed similar effects, namely improved insulin sensitivity and amelioration of hyperglycemia in mouse models of obesity, diabetes, and lipoatrophy, although following systemic infusion of physiological doses of the globular domain of adiponectin, not the full-length form.

Development of hyperinsulinemia is one possible mechanism for the suppression of adiponectin levels seen in these studies. However, hyperinsulinemia per se seems unlikely as a mediator of low adiponectin levels, since adiponectin levels remain low in the later stages of type 2 diabetes in association with decreased circulating insulin levels. Adipocyte insulin action or signal transduction rather than absolute levels of insulin may regulate adiponectin secretion. In support of this contention, Bogan and Lodish[120] have shown that secretion of adiponectin by 3T3-L1 adipocytes requires phosphatidylinositol 3-kinase (PI-3K), a major intermediate of insulin signaling activity. Insulin stimulated insulin receptor substrate 1 (IRS-1)-associated PI-3K activity has been shown to be decreased in adipocytes of type 2 diabetic subjects.[121]

Thus it is possible that the decreased adipocyte PI-3K activity in type 2 diabetic patients may contribute to the decreased adiponectin levels. Additional investigations to test this hypothesis are warranted. Other investigators have presented data on the potential inverse relationship between adiponectin and insulin action. Euglycemic-hyperinsulinemic clamp studies in both humans and rats[122] have shown that insulin infusion leads to decreased circulating adiponectin levels, consistent with the interpretation that insulin exerts an acute effect on adipocytes to decrease production and/or secretion of this adipocytokine.

There is published data[123] supporting a possible role of adiponectin in catecholamine-induced insulin resistance. They found that treating 3T3-L1 adipocytes with the adrenergic agonist isoproterenol reduced the level of adiponectin mRNA by 75% in vitro. This inhibitory effect of isoproterenol was almost completely reversed by pretreatment of the cells with the adrenergic antagonist propranolol and the protein kinase A (PKA) inhibitor H-89. The authors concluded that catecholamines might induce insulin resistance at least partly by downregulation of adiponectin gene expression, and that this inhibitory effect was mediated via adrenergic receptors through a Gs protein (stimulatory guanine nucleotide binding) PKA–dependent pathway. Homozygous (adipo adiponectin-deficient mice have been shown to have significantly increased insulin resistance when compared with wild-type and heterozygous (adipo) adiponectin-deficient mice in certain studies.[124] This loss-of-function experiment provides further evidence that adiponectin is indeed required for normal regulation of insulin sensitivity and glucose homeostasis in vivo.

The connection between adiponectin levels and insulin resistance has been further confirmed by data obtained from treatment with TZDs. The peroxisome proliferator–activated receptor (PPAR) gamma is a ligand-activated transcription factor thought to be a master regulator of adipocyte differentiation and multiple adipocyte genes. TZDs are specific synthetic ligand activators of PPAR-gamma that improve glucose tolerance and insulin sensitivity in type 2 diabetic patients and in animal models of insulin resistance through mechanisms that are incompletely understood. The administration of TZDs has been shown to increase the plasma adiponectin concentrations in insulin resistant humans and rodents and in subjects with type 2 diabetes.[103]

The promoter activity of the adiponectin gene has been shown to be markedly enhanced by the TZDs,[103] although the presence of a functional PPAR-gamma response element in the adiponectin gene remains controversial.[125,126] The induction of adiponectin in fact might be caused by secondary effects involving other PPAR inducible genes and not by specific activation of the PPAR response elements.[127] In support of an important role for PPAR-gamma in regulation of adiponectin synthesis, circulating adiponectin levels were found to be suppressed fivefold in patients with severe insulin resistance in association with dominant-negative PPAR- gamma mutations.[128] Thus, induction of adipose tissue adiponectin expression and consequent increases in circulating adiponectin levels could potentially represent a novel potential mechanism for PPAR-mediated enhancement of whole body insulin sensitivity.

Furthermore, adiponectin may be a biomarker of in vivo PPAR- gamma activation. Studies reported an increase in adiponectin levels in normal subjects

after only 14 days of treatment with rosiglitazone. This finding was supported by a recent study in rats, which showed a similar increase in adiponectin levels after two weeks of TZD treatment.[129]

The activation of PPAR-gamma by TZDs may promote weight gain by increasing adipocyte differentiation and the number of small adipocytes, as has been previously shown,[130] as well as enhance adiponectin gene transcription in existing mature adipocytes, thus increasing adiponectin levels. Adiponectin has also been proposed by some investigators as a reliable marker for insulin resistance in type 2 diabetes.

Researchers[131] used the hyperinsulinemic- euglycemic clamp to quantify glucose infusion rate (GIR) as an index for insulin sensitivity in 16 patients with type 2 diabetes. GIR was most strongly correlated with circulating adiponectin levels and fasting plasma glucose. The role of adiponectin in mitigating insulin resistance has been further substantiated by studies in humans and mice with lipodystrophies.[100]

Lipodystrophies are characterized by selective but variable loss of body fat and insulin resistance. Serum adiponectin levels are extremely low in patients with generalized lipodystrophies and may be related to the general absence of adipose tissue and/or associated severe insulin resistance. Researchers[100] showed that treating lipoatrophic mice with physiological doses of adiponectin significantly but not completely ameliorated hyperglycemia and hyperinsulinemia. Adipose tissue expression and circulating adiponectin concentrations have also been found to be significantly decreased in HIV-positive

patients with lipodystrophy treated with highly active antiretroviral therapy. Both serum and mRNA concentrations of adiponectin were found to closely correlate with features of insulin resistance, including hepatic fat content.[132] Thus, it may be reasonable to surmise that decreased production of adiponectin in lipoatrophic adipose tissue may contribute to the development of insulin resistance in these patients. Although a cause-and-effect association has not been definitely established, available evidence indicates that visceral fat is an important link between the many facets of the metabolic syndrome, including glucose intolerance, hypertension, dyslipidemia, and insulin resistance.[133]

Visceral adiposity is characterized by enhanced lipolysis[90] and augmented plasma FFA flux, especially into the portal circulation. Increased inflow of FFAs into the liver from the portal circulation is thought to retard insulin clearance and to enhance lipid synthesis, which may result in peripheral hyperinsulinemia and hyperlipidemia. FFAs have also been shown to induce hepatic insulin resistance by inhibiting insulin suppression of glycogenolysis during euglycemic hyperinsulinemic clamp studies[134] and to directly stimulate glycogenolysis and gluconeogenesis, thus contributing tomild fasting hyperglycemia in euglycemic subjects given lipid infusions.[135]

Adiponectin mRNA and protein levels have been found to be reduced in omental fat compared with subcutaneous fat.[136] Visceral fat may also produce an as-yet-unidentified factor that destabilizes adiponectin mRNA.[137] The strong inverse correlation between serum adiponectin levels and intra-abdominal fat mass may in part underlie the link between visceral fat and insulin resistance. Although these epidemiological and experimental studies are suggestive of a role

for adiponectin in insulin sensitivity and firmly establish an association between insulin resistance and low plasma adiponectin levels, it is not yet established whether decreased adiponectin levels are the cause or effect of this dysregulated metabolic state.

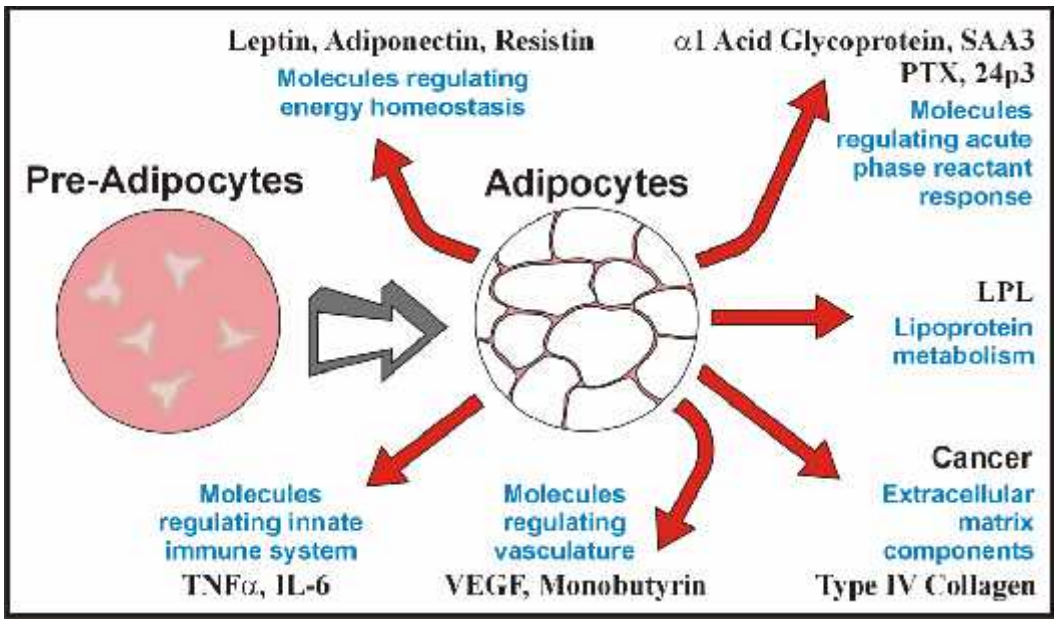


Figure 7. The role of adipocytes in energy homeostasis, immune system function and cancer.

Adipose tissue plays a major role in problems associated with prolonged hyperglycemia. Hyperglycemia induces a production of acute phase reactants in adipose tissue, including alpha 1 acid-glycoprotein, serum amyloid A3 (SAA3), lipocalin (24p3), paclitaxel (PTX). The adipocytes produce vascular endothelial growth factor (VEGF) in response to insulin and catecholamines. Monobutyrin (1-butyryl-glycerol) is a novel angiogenic and vasoactive factor secreted by differentiating adipocytes. LPL-lipoprotein lipase. Type VI collagen, a soluble

extracellular matrix protein abundantly expressed in adipocytes, is upregulated in adipocytes during tumorigenesis

Adiponectin and atherosclerosis

Experimental studies have indicated that adiponectin has potential antiatherogenic and anti-inflammatory properties.[105-107,138-141] Monocyte adhesion to the vascular endothelium and subsequent differentiation to macrophages and foam cells is considered crucial for the development of vascular disease.

In certain studies[19] it was found that adiponectin had effects on monocyte adhesion to endothelium, myeloid differentiation, and macrophage cytokine production and phagocytosis. Adiponectin has been shown to inhibit both the production and action of TNF-alpha, a cytokine that has direct effects on the adhesion molecules.[92,105] Although its receptor has not been identified, adiponectin modulates signaling of nuclear factor beta (NF Beta) (a transcription factor involved in the inflammatory response), at least partly through a cAMP-dependent pathway.[138] it has been shown that adiponectin suppressed macrophage to foam cell transformation in vitro.[139] Thus adiponectin probably serves as a modulator for macrophage foam cell formation and could provide an answer to the fundamental mechanism for the link between vascular inflammation and atherosclerosis.

Furthermore, adiponectin mediated signaling has been shown to inhibit growth factor–induced human aortic smooth muscle cell proliferation and migration.[106] These in vitro studies demonstrate that adiponectin may act as an antiatherosclerotic factor through a direct effect on endothelial cells. Severe

neointimal thickening and increased proliferation of vascular smooth muscle cells has been demonstrated in mechanically injured arteries of adiponectin knockout mice. Supplementation of adiponectin in this mouse model attenuated the neointimal proliferation.[140]

This has been the first in vivo evidence that adiponectin might serve as a critical link bridging the adipose tissue–vascular axis. Amelioration of atherosclerosis associated with decreased expression of class A scavenger receptor and TNF-alpha has been demonstrated in globular adiponectin transgenic (gAd Tg) apo E–deficient mice.[116] This appears to be the first in vivo demonstration of a protective role of adiponectin against atherosclerosis. High-sensitive C-reactive protein (hs-CRP) is a well-known marker and risk factor for coronary artery disease. It was recently shown that CRP mRNA is expressed in human adipose tissue.[141]

A significant inverse correlation has been observed between CRP and adiponectin mRNA levels in subcutaneous adipose tissue of human subjects with angiographically demonstrated coronary atherosclerosis.[141] The same negative correlation exists between plasma hs-CRP and adiponectin levels. This reciprocal association between adiponectin and CRP levels in both human adipose tissue and plasma is supportive of a role for adiponectin against the development of atherosclerosis and vascular inflammation.

Chapter 4

Methodology

METHODOLOGY

This study was done at Department of Nephrology, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

Study design

A one year cross sectional study.

Study period and duration

One year, from January 2012 to December 2012.

Place

This study was conducted in the Department of Medicine/Nephrology, 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 type 2 diabetes mellitus and chronic kidney disease admitted at Department of Medicine/Nephrology, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the study period were included in the study.

Sample size

A total of 100 patients with type 2 diabetes mellitus and chronic kidney disease were selected for the study.

Sampling procedure

Based on the literature for CKD among type 2 diabetics the sample size was calculated using the following formula as below.

$n = 4 p q / d^2$

Where, p = Prevalence (50%)

q = 100 – p = 50%

d = Absolute error considered as 10%

Hence,

$n = 4 \times 50 \times 50 / 100^2$

n = 100

Hence a sample size of 100 patients with type 2 diabetes mellitus and chronic kidney disease was considered

Selection criteria

Inclusion

- Patients of type 2 diabetes mellitus with chronic kidney disease stage two, three and four.

Exclusion

- Type 1 diabetic patients.
- Patients on haemodialysis.
- Recipients of renal transplants
- Patients with;
    - Active infection or inflammation.

- Hypertriglyceridemia.
- Smoking habit.

- Patients on;
  - Thiazolidindiones oral hypoglycemics.
  - Statin therapy.

Ethical clearance

Prior to the commencement, the ethical clearance was obtained from Institutional Ethics Committee, Jawaharlal Nehru Medical College, Belgaum.

Informed Consent

The patients willing to participate in the study were enrolled after obtaining a written informed consent (Annexure I).

Method of collection of data

Demographic data such as age, sex and occupation were recorded. Patients were interviewed and history regarding type 2 diabetes mellitus such as duration of disease, symptoms and other comorbid conditions were evaluated. A thorough physical examination was conducted for anthropometry, vitals (pulse rate, blood pressure and respiratory rate) and clinical signs followed by systemic examination including fundoscopy. These findings were recorded on a predesigned and pretested proforma (Annexure II).

Investigations

The patients were evaluation for following laboratory markers.

- Random blood sugar.
- Glycated haemoglobin (HbA1c).
- Urine – Routine and microscopy.
- Serum triglyceride.
- Serum creatinine.
- C-reactive protein.
- Serum adiponectin (ELISA)

Outcome variables

Body mass index

A thorough clinical examination was conducted. Height and weight was recorded and body mass index was calculated based on formula;

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

Body mass index was classified according to Overweight and obesity by BMI in adult Asians as below.[142]

Classification	BMI (Kg/m^2)	Risk of co-morbidities
Underweight	< 18.5	Low (But increased risk of other clinical problems
Normal range	18.5 to 22.9	Average
Overweight	≥ 23	
At risk	23.0 to 24.9	Increased
Obese I	25.0 to 29.9	Moderate
Obese II	≥ 30.0	Severe

Urine protein estimation

Urine Micro-Albumin was done by immunoturbidometry. 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

Serum triglyceride

Triglycerides were noted and less 150 mg/dL was interpreted as normal based on NCEP (National Cholesterol Education Program) guidelines.[143]

Serum Creatinine (to calculate eGFR by MDRD Formula)

Serum creatinine estimation was done by Jaeffe's method and values between 0.8 mg/dL to 1.3 mg/dL were interpreted as normal.

eGFR

eGFR was calculated using the formula as below.

$eGFR = 1.86 \times Serum\ Creatinine^{-1.154} \times Age^{-0.203}$

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

Adiponectin[144]

Blood samples for measuring fasting plasma adiponectin were drawn under all aseptic precautions and blood was collected in plain tubes. All tubes were cold centrifuged (4^oC) within several minutes of collection and stored at 70^oC until assay at the Department of Biochemistry. The estimation of serum

adiponectin was done by ELISA method and the values obtained were interpreted as below.

- 8.3 µg/mL or less
- 8.4 to 15.1 µg/mL
- > 15.1 µg/mL

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

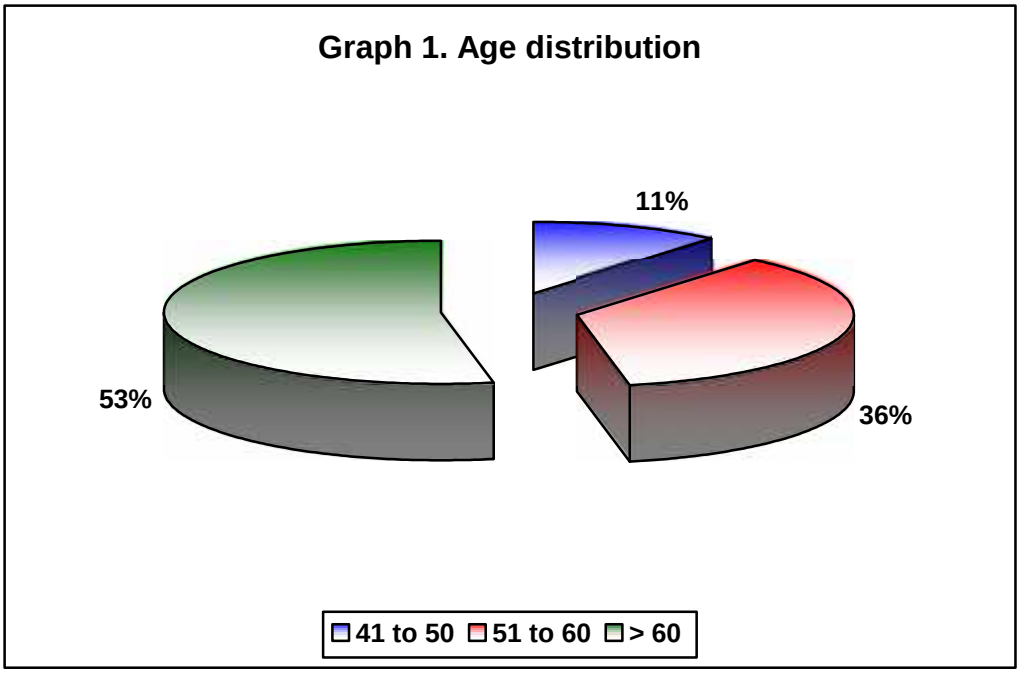
Results

RESULTS

In this one year cross sectional study done in the in the Department of Medicine/Nephrology, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum, during the study period from January 2012 to December 2012, a total of 100 patients with type 2 diabetes mellitus with chronic kidney disease admitted were studied and the findings / observations are tabulated as below.

Table 1. Age distribution

Age group (Years)	Distribution (n=100)	
	Number	**Percentage**
41 to 50	11	11.00
51 to 60	36	36.00
> 60	53	53.00
Total	**100**	**100.00**

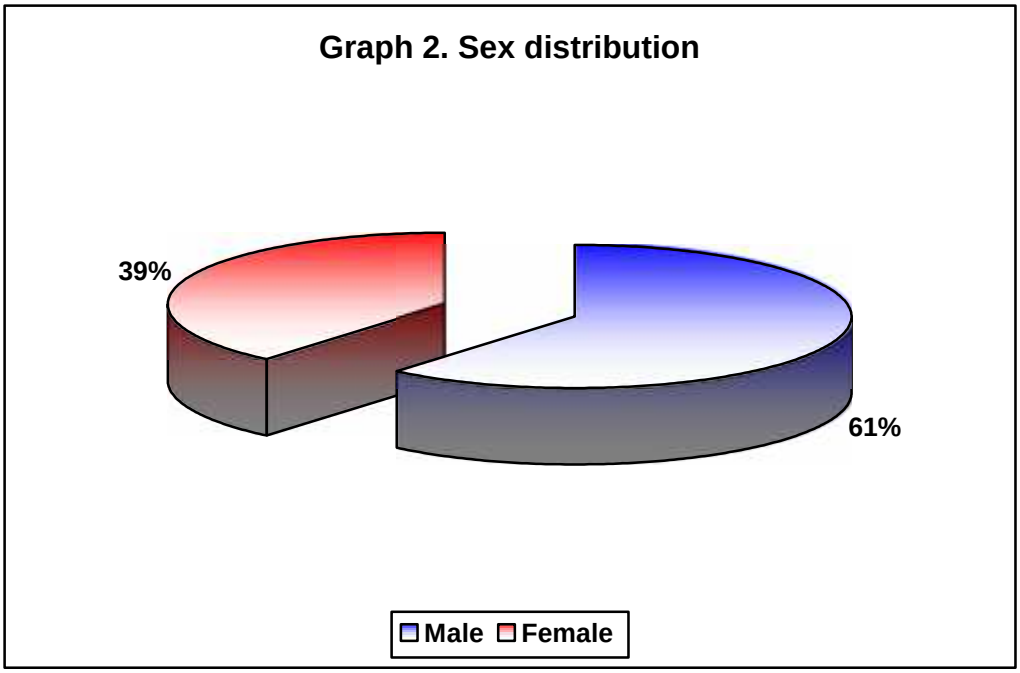


Patient's age ranged from 41 to 91 years, maximum patients were in the age group of >60 years i.e. 53 (53%), between 51 to 60 years 36 patients (36%) and between 41 to 50 11 patients (11%). Mean age was 64.4 ± 10.23 years.

Inference : Maximum number of cases were in the age group of >60 years.

Table 2. Sex distribution

Sex	Distribution (n=100)	
	Number	**Percentage**
Male	61	61.00
Female	39	39.00
Total	**100**	**100.00**

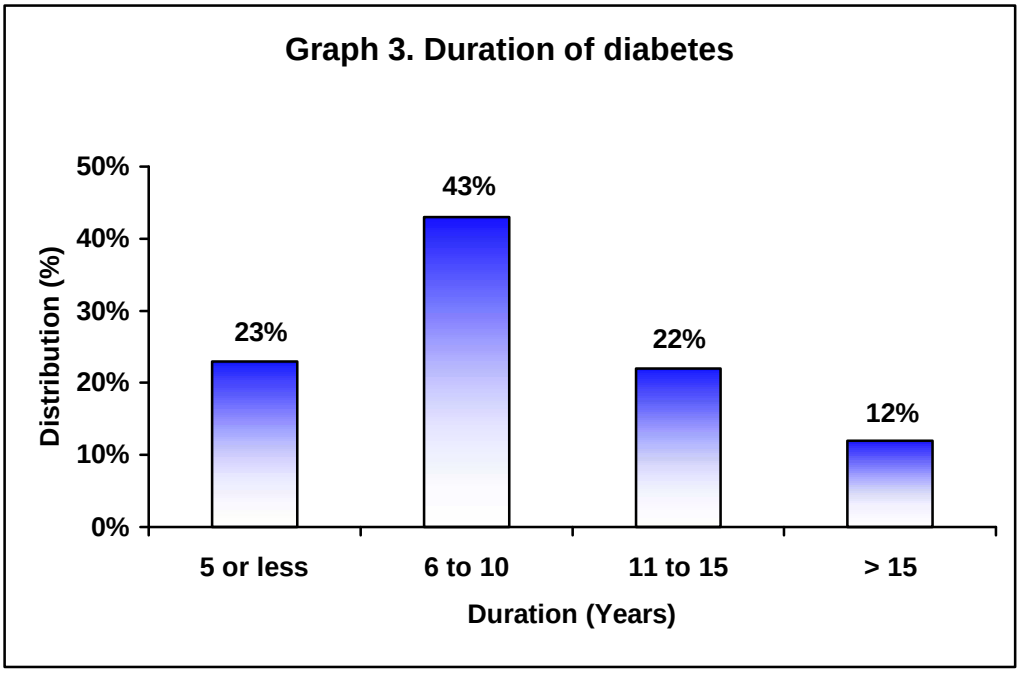


Out of 100 patients 61 patients (61%) were males and remaining 39 patients (39%) were females, accounting a ratio of male to female 1.56 to 1.

Inference: Male preponderance was observed.

Table 3. Duration of diabetes

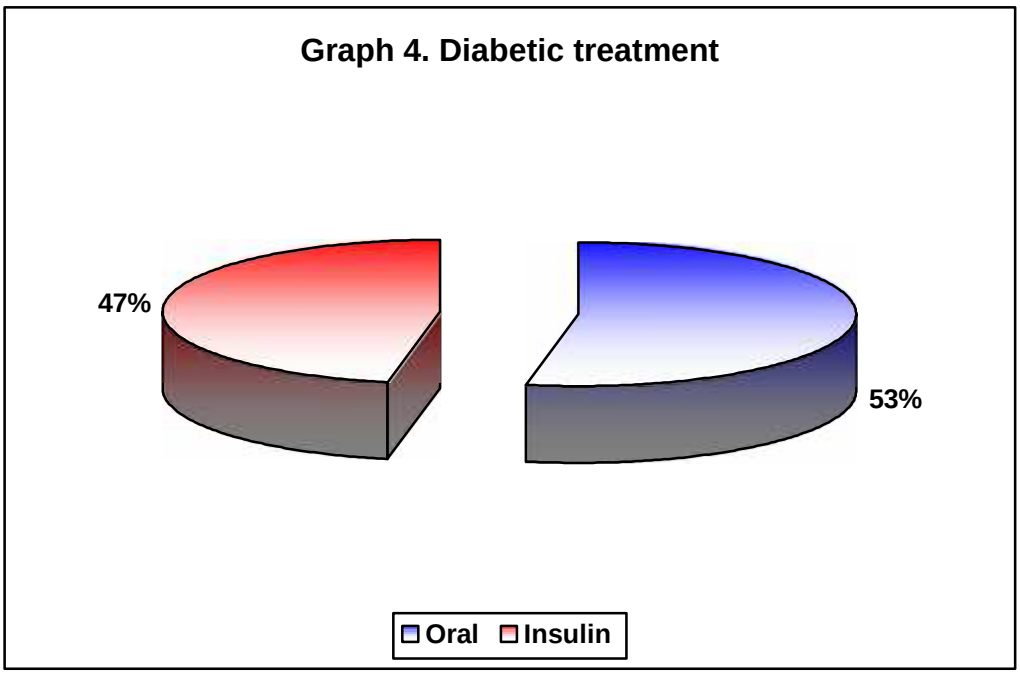
Duration (years)	Distribution (n=100)	
	Number	Percentage
≤5	23	23.00
6 to 10	43	43.00
11 to 15	22	22.00
> 15	12	12.00
Total	**100**	**100.00**



We observed in 43 (43%) patients the duration of diabetes was 6 to 10 years, 22 patients (22%) the duration was between 11 to 15 years and in 12 (12%) patients the duration was >15 years. In 23 patients (23%) the duration was upto 5 years.

Table 4. Diabetic treatment

Treatment	Distribution (n=100)	
	Number	**Percentage**
Oral Hypoglycemic Agents	53	53.00
Insulin	47	47.00
Total	**100**	**100.00**



53 patients (53%) were on oral hypoglycemic agents and remaining 47 (47%) were on insulin preparations.

Table 5. Co-morbid conditions - Hypertension

Blood Pressure	Distribution (n=100)	
	Number	**Percentage**
Hypertension	27	27.00
Normal	73	73.00
Total	**100**	**100.00**

27 patients (27%) had associated hypertension, remaining 73 patients (73%) were normotensives.

Table 6.Family history of chronic kidney disease with type 2 diabetes mellitus

Chronic Kidney Disease	Distribution (n=100)	
	Number	**Percentage**
With CKD	40	40.00
Without CKD	60	60.00
Total	**100**	**100.00**

In 40 (40%) patients there was a strong family history of chronic kidney disease with type 2 diabetes mellitus, remaining 60 (60%) there was no family history of chronic kidney disease.

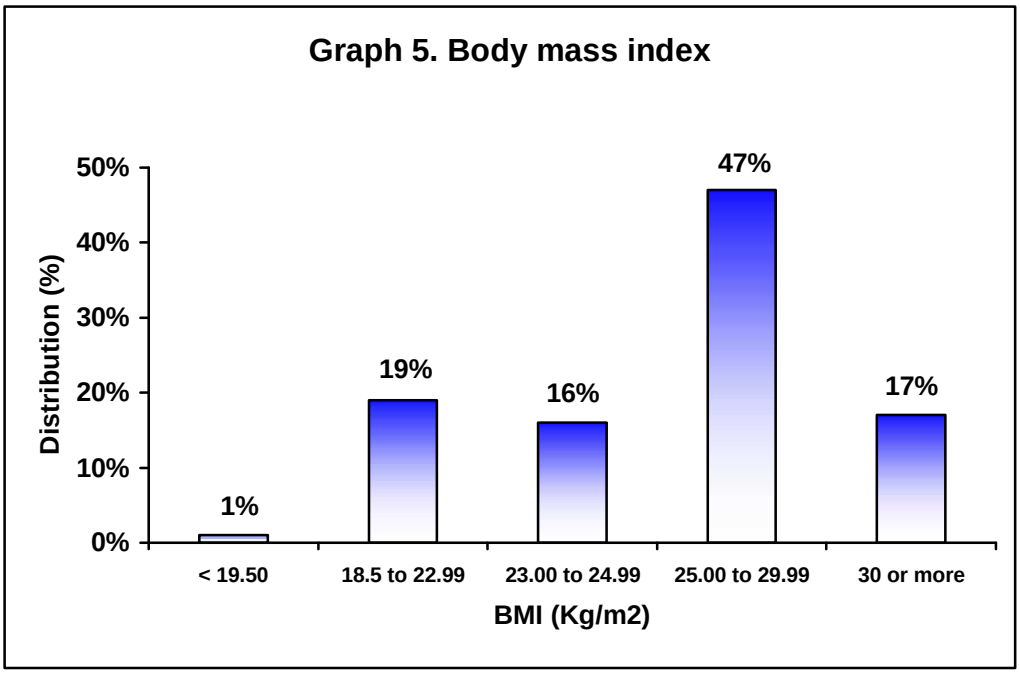
Table 7. Clinical presentations

Symptoms	Distribution (n=100)	
	Number	**Percentage**
Dyspnea	0	0
Dyspepsia	6	6.00
Pain abdomen	4	4.00
Oliguria	11	11.00
Dyspepsia and Oliguria	5	5.00
Dyspepsia and Pain abdomen	2	2.00
Dyspnea and Oliguria	2	2.00
Dyspnea, Dyspepsia and Oliguria	1	1.00
All of the above	1	1.00
None of the above	68	68.00
Total	**100**	**100**

Patients presented with symptoms pertaining to chronic kidney disease, the commonest symptom was oliguria in 11% followed by dyspnea 6% and 4% with pain abdomen. 11 patients (11%) presented with overlapping symptoms. 68% of the patients did not have any symptoms.

Table 8. Body mass index

Body mass index (Kg/m2)	Distribution (n=100)	
	Number	Percentage
< 18.50	1	1.00
18.5 to 22.99	19	19.00
23.00 to 24.99	16	16.00
25.00 to 29.99	47	47.00
30 or more	17	17.00
Total	**100**	**100.00**



In 80 patients (80%) BMI varied between 23 to >30, in 19 patients (19%) BMI was within normal limits and in only 1 patient (1%) BMI was below normal.

Table 9. Clinical observations

Signs	Distribution (n=100)	
	Number	**Percentage**
Pallor	36	36.00
Oedema	7	7.00
Both	17	17.00
No signs (Pallor or Oedema)	40	40.00
Total	**100**	**100.00**

The commonest observation on examination was pallor 36%, oedema 7% and both signs together in 17%. 40% did not have these signs.

Table 10. Associated complications - Diabetic retinopathy

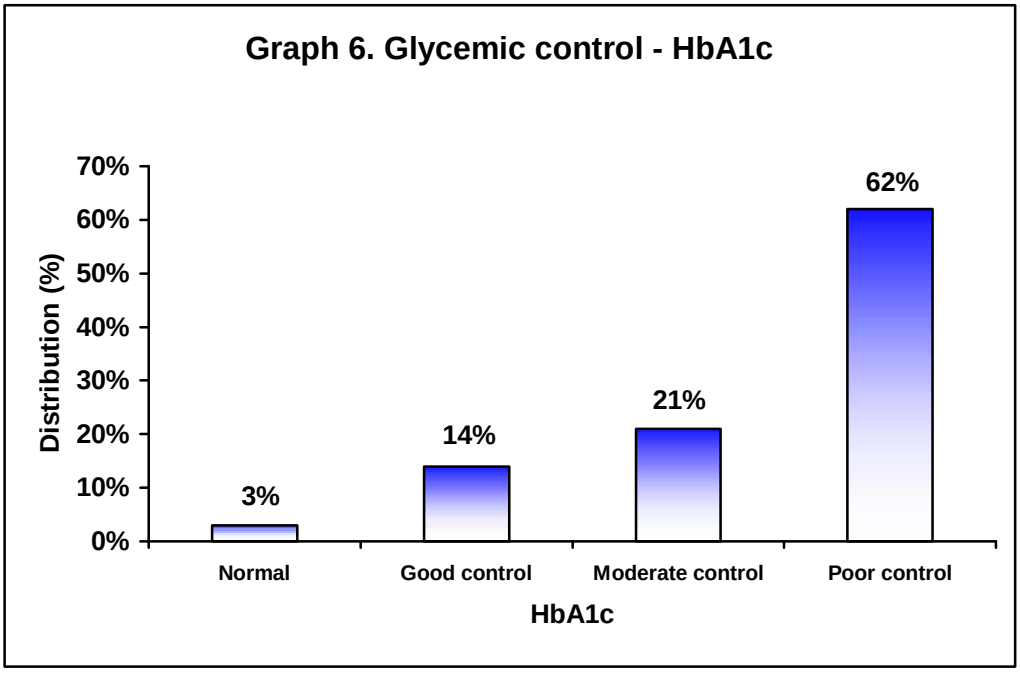
Diabetic retinopathy	Distribution (n=100)	
	Number	**Percentage**
Retinopathy	60	60.00
No retinopathy	40	40.00
Total	**100**	**100.00**

In 100 patients of chronic kidney disease with type 2 diabetes mellitus 60 (60%) had associated diabetic retinopathy, remaining 40 (40%) did not have evidence of diabetic retinopathy.

LABORATORY PARAMETERS

Table 11. Glycated hemoglobin – [HbA1c]

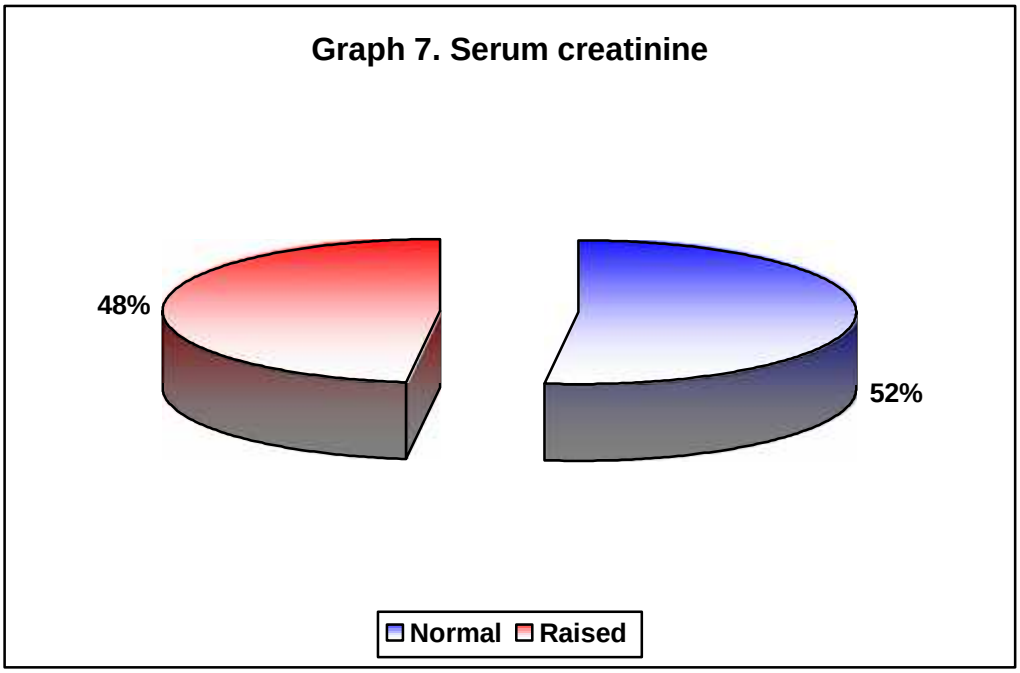
HbA1c (%)	Distribution (n=100)	
	Number	**Percentage**
Normal (4.7-6.4)	3	3.00
Good control (6.5-7.0)	14	14.00
Moderate control (7.1-8.0)	21	21.00
Poor control (>8.0)	62	62.00
Total	**100**	**100.00**



In 83 patients (83%) we observed the abnormality of HbA1c (>7.0%), remaining 17 patients (17%) , 14 were under good control and 3 had normal HbA1c.

Table 12. Serum creatinine

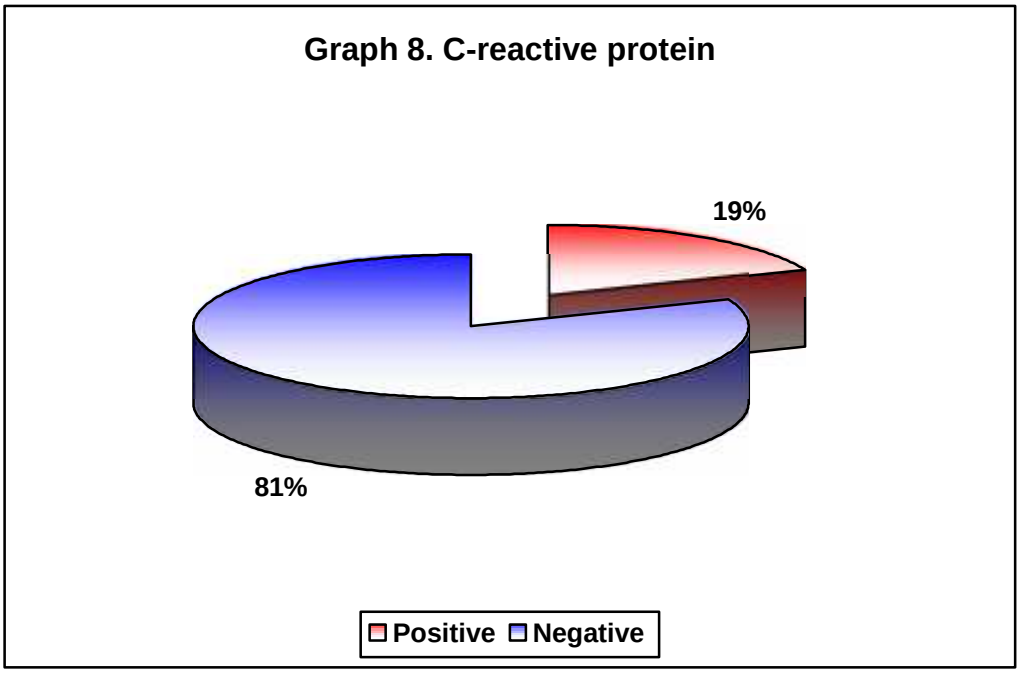
Serum creatinine (mg/dl)	Distribution (n=100)	
	Number	**Percentage**
Normal (0.8 to 1.3)	52	52.00
Raised (> 1.3)	48	48.00
Total	**100**	**100.00**



In 52 patients (52%) the serum creatinine was within normal limits, remaining 48 patients (48%) the levels were elevated.

Table 13. C–reactive protein

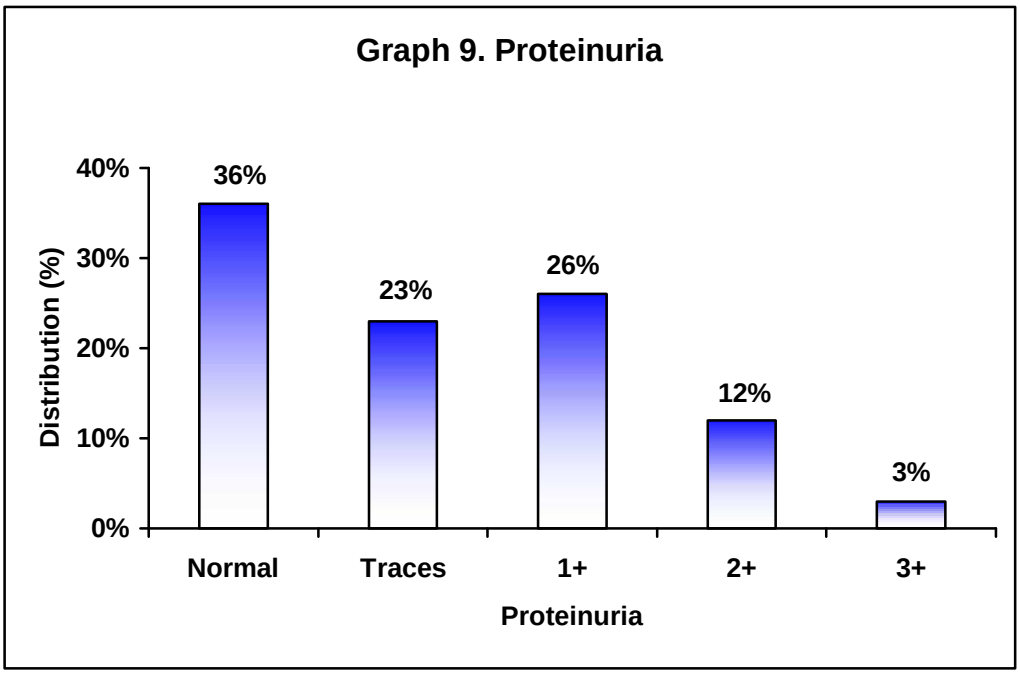
C-reactive protein	Distribution (n=100)	
	Number	**Percentage**
Positive	19	19.00
Negative	81	81.00
Total	**100**	**100.00**



In 81 patients (81%) C-reactive protein was negative and in 19 patients (19%) it was positive.

Table 14. Proteinuria

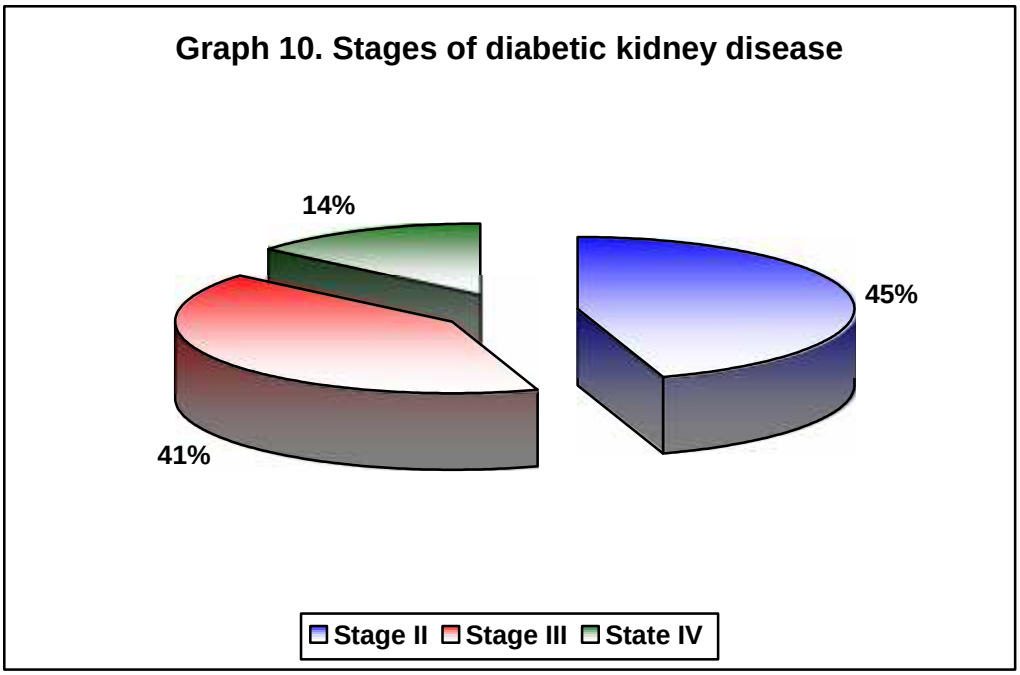
Proteinuria	Distribution (n=100)	
	Number	**Percentage**
Normal	36	36.00
Traces	23	23.00
1+	26	26.00
2+	12	12.00
3+	3	3.00
Total	**100**	**100.00**



Proteinuria was observed in 64 patients (64%) in varying proportions, ranging from traces to gross proteinuria, remaining 36 patients (36%) however there was no proteinuria.

Table 15. Stages of chronic kidney disease

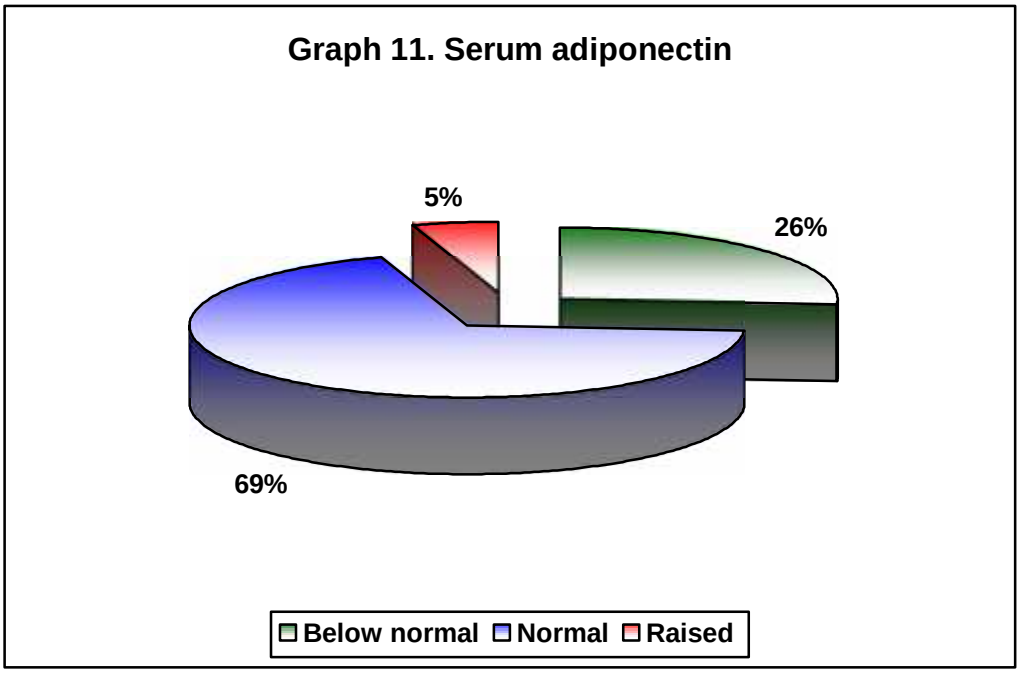
Stage/eGFR(ml/min)	Distribution (n=100)	
	Number	Percentage
Stage II (60 to 89)	45	45.00
Stage III (30 to 59)	41	41.00
Stage IV (15 to 29)	14	14.00
Total	**100**	**100.00**



Maximum number of patients were in the stage of II to III chronic kidney disease [86 patients (86%)]. Only 14 patients(14%) were in stage IV chronic kidney disease.

Table 16. Serum adiponectin

Serum adiponectin (µg/ml)	Distribution (n=100)	
	Number	Percentage
Below normal (8.3 or less)	26	26.00
Normal (8.4 to 15.1)	69	69.00
Raised (15.2 or more)	5	5.00
Total	**100**	**100.00**



In 69 patients (69%) of chronic kidney disease the levels of adiponectin were within normal limits, in 26 patients (26%) the levels were below normal and in only 5 patients (5%) they were more than normal.

Table 17. Comparison of serum adiponectin levels with age

Age group (Years)	Serum adiponectin (µg/mL)						Total (n=100)	
	8.3 or less		8.4 to 15.1		≥ 15.2			
	No	%	No	%	No	%	No	%
41 to 50	1	9.09	10	90.91	0	0.00	11	100.00
51 to 60	6	16.67	30	83.33	0	0.00	36	100.00
> 60	19	35.85	29	54.72	5	9.43	53	100.00
Total	**26**	**26.00**	**69**	**69.00**	**5**	**5.00**	**100**	**100.00**

p = 0.017

In patients aged >60 years the levels were less than normal [19 patients (35.85%)], in 6 patents (16.67%) between ages of 51 to 60 years the levels were also less. In only 1 patient (9.09%) who was between 41 to 50 years the level was low.

In 69 patients the levels were within normal limits [>60 yrs:- 29 patients (54.72%); 51 to 60 yrs:- 30 patients(83.33%); 41 to 50 yrs:- 10 patients (90.91%)].

In 5 patients (9.43%) the levels were more than normal, all these patients were more than 60 years of age.

[p value = 0.017 – statistically significant]

Table 18. Comparison of serum adiponectin levels with Gender

Sex	Serum adiponectin (µg/ml)						Total (n=100)	
	8.3 or less		8.4 to 15.1		≥ 15.2			
	No	%	No	%	No	%	No	%
Male	17	27.87	44	72.13	0	0.00	61	100.00
Female	9	23.08	25	64.10	5	12.82	39	100.00
Total	**26**	**26.00**	**69**	**69.00**	**5**	**5.00**	**100**	**100.00**

p = 0.016

In 26 patients (26%) [Male:- 17 (27.87%); Female:- 9 (23.08%)] the levels were below normal, in 69 patients (69%) [Male:- 44 (72.13%); Female:-25 (64.10%)] the levels were within normal limits. Levels more than normal were observed only in 5 female patients (12.82%).

[p value = 0.016 – statistically significant]

Table 19. Comparison of serum adiponectin with body mass index(BMI)

BMI (Kg/m^2)	Serum adiponectin (µg/mL)						Total (n=100)	
	8.3 or less		8.4 to 15.1		≥ 15.2			
	No	%	No	%	No	%	No	%
< 18.50	1	100.00	0	0.00	0	0.00	1	100.00
18.5 to 22.99	3	15.79	15	78.95	1	5.26	19	100.00
23.00 to 24.99	4	25.00	11	68.75	1	6.25	16	100.00
25.00 to 29.99	14	29.79	31	65.96	2	4.26	47	100.00
30 or more	4	23.53	12	70.59	1	5.88	17	100.00
Total	**26**	**26.00**	**69**	**69.00**	**5**	**5.00**	**100**	**100.00**

p = 0.744

Adiponectin levels when compared with BMI, 22 patients having BMI of >23 had low levels [23.00 to 24.99:- 4 patients (25%); 25.00 to 29.99:- 14 patients (29.79%); >30:- 4 patients(23.53%)]. In 3 patients (15.79%) with normal BMI, levels were low and in 1 patient the BMI was below normal with low levels.

In 54 patients whose BMI ranged from 23 to ≥30 the adiponectin levels were within normal limits [23.00 to 24.99:- 11 patients (68.75%); 25.00 to 29.99:- 31 patients (65.96%); >30:- 12 patients (70.59%)]. In 15 patients (78.95%) with normal BMI levels were within normal limits.

5 patients had levels more than normal whose BMI ranged from 18.5 to ≥30 [18.5 to 22.99:- 1 patient(5.26%); 23.00 to 24.99:- 1 patient(6.25%); 25.00 to 29.99:- 2 patients(4.26%); >30:- 1 patient(5.88%)].

[p value = 0.744 – statistically non significant]

Table 20. Comparison of serum adiponectin levels with HbA1c

HbA1c (%)	Serum adiponectin (µg/ml)						Total (n=100)	
	8.3 or less		8.4 to 15.1		≥ 15.2			
	No	%	No	%	No	%	No	%
4.7-6.4	0	0.00	3	100.00	0	0.00	3	100.00
6.5-7.0	6	42.86	7	50.00	1	7.14	14	100.00
7.1-8.0	6	30.00	14	70.00	0	0.00	20	100.00
>8.0	14	22.22	45	71.43	4	6.35	63	100.00
Total	**26**	**26.00**	**69**	**69.00**	**5**	**5.00**	**100**	**100.00**

p = 0.493

In 20 patients with HbA1c between 7.1% to ≥8.0% [7.1% to 8.0%:- 6 patients (28.57%); >8.0%:-14 patients (42.42%)] the levels were low. In 6 patients (42.86%) HbA1c was 6.5% to 7.0% with low levels.

In 59 patients the levels were within normal limits, their HbA1c was 7.1% to ≥8.0% [7.1% to 8.0%:- 14 patients (70.00%); >8.0%:- 45 patients (71.43%)]. Remaining 3 patients (100%) and 7 patients (50%) with normal levels had HbA1c of 4.7 to 6.4 and 6.5 to 7.0 respectively.

5 patients had elevated levels of adiponectin [HbA1c:6.5% to 7.0%:- 1 patient (7.14%); >8.0%:- 4 patients (6.35%)].

[p value = 0.493 – statistically non significant]

Tale 21. Comparison of serum adiponectin levels with serum creatinine levels

Serum creatinine (mg/dL)	Serum adiponectin (µg/mL)						Total (n=100)	
	8.3 or less		8.4 to 15.1		≥ 15.2			
	No	%	No	%	No	%	No	%
0.8 to 1.3	4	7.69	46	88.46	2	3.85	52	100.00
> 1.3	22	45.83	23	47.92	3	6.25	48	100.00
Total	**26**	**26.00**	**69**	**69.00**	**5**	**5.00**	**100**	**100.00**

p < 0.001

In 4 patients (7.69%) serum creatinine was normal with low levels of adiponectin. In 22 patients (45.83%) with low levels the creatinine was elevated.

In 46 patients (88.64%) with normal levels of adiponectin creatinine was also normal (0.8 to 1.3). 23 patients (47.92%) in whom creatinine was elevated (>1.3) the levels were within normal limits.

In remaining 5 patients , in 2 (3.85%) with creatinine being normal and other 3 (6.25%) creatinine being elevated the levels were more.

[p value < 0.001 – statistically significant]

Table 22. Comparison of serum adiponectin with proteinuria

Proteinuria	Serum adiponectin (µg/mL)						Total (n=100)	
	8.3 or less		8.4 to 15.1		≥ 15.2			
	No	%	No	%	No	%	No	%
Normal	10	27.78	24	66.67	2	5.56	36	100.00
Traces	1	4.35	21	91.30	1	4.35	23	100.00
1+	10	38.46	15	57.69	1	3.85	26	100.00
2+	5	41.67	7	58.33	0	0.00	12	100.00
3+	0	0.00	2	66.67	1	33.33	3	100.00
Total	**26**	**26.00**	**69**	**69.00**	**5**	**5.00**	**100**	**100.00**

p = 0.033

10 patients out of 26 patients who had no proteinuria had low levels of adiponectin, remaining 16 patients had proteinuria ranging from traces to 3+ [Traces:- 1 patient(4.35%); 1+:- 10 patients(38.46%); 2+:- 5 patients(41.67%); 3+:- 0 patient] had low levels.

In 24 patients without proteinuria the levels were within normal limits, remaining 45 patients with proteinuria ranging from traces to 3+ [Traces:- 21 patient(91.30%); 1+:- 15 patients(57.69%); 2+:- 7 patients(58.33%); 3+:- 2 patients(66.67%)] the levels were normal.

In 5 patients the levels were more than normal, 2 patients(5.56%) were without proteinuria, remaining 3 had proteinuria, 1(4.35%) had traces, 1(3.85%) had 1+ and 1(33.33%) had 3+.

[p value = 0.033 – statistically significant]

Table 23. Comparison of serum adiponectin levels with stages of chronic kidney disease

Stages	Serum adiponectin (µg/mL)						Total (n=100)	
	8.3 or less		8.4 to 15.1		≥ 15.2			
	No	%	No	%	No	%	No	%
II	3	6.67	41	91.11	1	2.22	45	100.00
III	17	41.46	23	56.10	1	2.44	41	100.00
IV	6	42.86	5	35.71	3	21.43	14	100.00
Total	**26**	**26.00**	**69**	**69.00**	**5**	**5.00**	**100**	**100.00**

p < 0.001

In all 26 patients who had low levels of adiponectin had evidence of chronic kidney disease [stage II:- 3 patients(6.67%); stage III:- 17 patients (41.46%); stage IV:- 6 patients(42.86%)].

In 69 patients with normal levels of adiponectin the staging varied [stage II:- 41 patients (91.11%); stage III:- 23 patients (56.10%); stage IV:- 5 patients (35.71%)].

In remaining 5 patients the levels of adiponectin were more than normal, 1 patient in each group of stage II (2.22%) and stage III (2.44%) respectively, and 3 patients in stage IV (21.43%).

[p value <0.001 – statistically significant]

Chapter 6

Discussion

DISCUSSION

In present study of 100 patients association of serum Adiponectin levels with stage 2 to 4 chronic kidney disease in patients of type 2 diabetes mellitus, we compared the levels of Adiponectin with various factors and the observations are as follows.

In our study patient's age ranged from 41 to 91 years. Maximum number of cases (53 patients) were in the age group of more than 60 years; 19 had low, 29 had normal and 5 had high levels of Adiponectin (p value = 0.017 - statistically significant). This is in contrast with the study done by Lin J et al[15] who found in diabetic individuals with increasing age the levels of Adiponectin were higher as compared to younger individuals.

A recent Japanese study by Obata Y et al[145] also found significantly higher levels of Adiponectin in older population of healthy as well as diabetic individuals which was independent of renal function.

Similar to the findings observed in our study, study by Menon V et al[146] observed low levels of Adiponectin in patients who were older as compared to younger but this observation was statistically insignificant.

Association of levels of Adiponectin in relation to gender, we observed low levels in 26 patients (Male:- 17 ; Female:- 9). In 69 patients (Male:- 44 ; Female:- 25) the levels were within normal limits. In 5 patients (all females) the levels were more than normal (p value = 0.016 - statistically significant). Here

females had higher levels of Adiponectin as compared to males. Similar observation was made by Menon V et al.[146]

Another study by Xu A et al[147] found elevated levels of Adiponectin in female gender. One more study by Saltevo J et al[148] also found higher Adiponectin levels in females across all levels of glucose tolerance compared to males.

Correlation of levels of Adiponectin with BMI, 26 patients who had low Adiponectin levels their BMI varied (<18.50 to >30). In significant proportion of patients (69%) the levels were though normal their BMI also varied (18.50 to >30). In 5 patients the levels were more than normal, all were females with BMI ranging from 18.50 to >30. P value = 0.744 - statistically insignificant. In our study there was no correlation between BMI and Adiponectin levels may be because of small sample size.

Menon V et al[146] found low levels with higher BMI. In their study p value was statistically significant, this difference could be because of their sample size being more.

Studies by Schulze MB et al[149] and Okauchi Y et al[150] also found statistically significant low levels of Adiponectin in patients with higher BMI.

Considering HbA1c comparison with levels of Adiponectin, we found no significant correlation between HbA1c and Adiponectin levels (p value = 0.493 - statistically insignificant), may be due to small sample size in our study. Other studies by Menon V et al,[146] Yilmaz MI et al[151] and Schulze MB et al[149] found

higher the HbA1c lower were the levels of Adiponectin and p value being statistically significant in their studies.

In one more study by Looker HC et al[152] the correlation did not show statistical significance though the sample size was more. This finding could be related to declined renal function and duration of diabetes in their study population as stated by the authors.

Levels of Adiponectin compared to serum creatinine, in our study we found higher creatinine levels were associated with low levels of Adiponectin (p value < 0.001 - statistically significant). Kacso IM et al[153] compared the levels of Adiponectin with declining renal function (follow up study), found low levels with worsening renal functions and predicted the low levels of Adiponectin were related to advancing kidney disease in patients of type 2 diabetes mellitus.

This is in sharp contrast with study done by Looker HC et al[152] who found statistically significant high levels of Adiponectin in patients with elevated serum creatinine.

An attempt to compare Adiponectin levels with proteinuria, we found that proteinuria was associated with low levels of Adiponectin. 16 patients out of 26 patients with low levels of Adiponectin had proteinuria. 45 patients though they had proteinuria ranging from traces to 3+, their levels of Adiponectin were within normal limits. 2 patients who did not have proteinuria the levels were more than normal and in 3 patients in spite of having proteinuria the levels were more than normal, p value = 0.033 - statistically significant.

Low levels of Adiponectin in patients of proteinuria observed in our study is similar to a study by Yilmaz MI et al[151] (p value < 0.001 – statistically significant).This observation is in contrast to study by Menon V et al[146] (p value = 0.01 – statistically insignificant).

In our study comparing the levels of Adiponectin with staging of chronic kidney disease, we found low levels of Adiponectin in patients with higher stage of chronic kidney disease (p value < 0.001- statistically significant). An observation by Lin J et al[15] found inverse relationship between renal dysfunction and levels of Adiponectin.

A study by Kacso IM et al[153] (follow up study) observed low levels of adiponectin with advancing chronic kidney disease in patients of type 2 diabetes mellitus.

Study by Menon V et al[146] does not support the above mentioned findings and observed elevated levels of Adiponectin in patients with reduced glomerular filtration rate and stated that further studies are required to understand the potential mechanism underlying this relationship.

Another study by Looker HC et al[152] observed that impaired renal function was associated with higher levels of Adiponectin in patients with type 2 diabetes mellitus, which is in contrast to the findings observed in present study.

We analyzed the data qualitatively and quantitatively to find out correlation of low levels of Adiponectin with chronic kidney disease in patients with type 2 diabetes mellitus, we found significant association when compared

with age, gender, creatinine, proteinuria and staging of chronic kidney disease. However, significant association between BMI, HbA1c and Adiponectin levels was not observed.

We feel it is necessary to study further to arrive at a stronger correlation with various variables in patients of type 2 diabetes mellitus having chronic kidney disease and to learn the possible mechanism underlying this relationship.

One limitation as far as our study is concerned is small sample size.

However, with this small sample size we conclude that progressive decline in renal function in patients with type 2 diabetes mellitus is related to the low levels of Adiponectin.

Chapter 7

Conclusion

CONCLUSION

In this study, patients of type 2 diabetes mellitus presenting with chronic kidney disease had lower levels of Adiponectin as the stage of chronic kidney disease varied from stage II to IV. Advanced stage of chronic kidney disease in patients with T2DM was associated with low levels of adiponectin as significantly higher number of patients with stage IV CKD (42.86%) had serum adiponectin levels ≤ 8.3 μg/mL compared to those having stage III (41.46%) and stage II (6.67%) ($p<0.001$).

Further the results showed significant association of low levels of serum adiponectin with age, gender, creatinine, proteinuria and stages of chronic kidney disease.

Chapter 8

Summary

SUMMARY

Adiponectin plays an important role in the pathogenesis of type 2 DM. The present study was aimed to find the association of serum adiponectin levels with stage 2 to stage 4 of Chronic Kidney Disease in patients with type 2 Diabetes mellitus.

The present one year cross sectional study was done at Department of Nephrology/Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. A total of 100 patients with type 2 diabetes mellitus and chronic kidney disease admitted during the study period from January 2012 to December 2012 were studied.

In this study, maximum patients were in the age group of >60 years (53%) and the mean age was 64.4 ± 10.23 years. Of the 100 patients, 61% were males and 39% were females with male to female ratio of 1.56 to 1. The duration of diabetes was 6 to 10 years in 43% of the patients. Maximum number of patients were in the stage of II to III chronic kidney disease (86%) and 14% were in stage IV chronic kidney disease. In 26% of the patients, the adiponectin levels were below normal and in 5% they were more than normal.

Advanced stage of the CKD in patients with T2DM was associated with low levels of adiponectin as higher number of patients with stage IV CKD (42.86%) had serum adiponectin levels ≤ 8.3 μg/mL. Low levels of serum adiponectin were also associated with age, gender, creatinine and proteinuria.

Chapter 9

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Annexures

Annexure I

ANNEXURE I – CONSENT FORM

"Association of Serum Adiponectin levels with Chronic Kidney Disease stage 2 to stage 4 in Patients with Type 2 Diabetes Mellitus."

Objective and purpose of the study:

This research is intended to estimate the Serum Adiponectin levels in patients with Diabetic Kidney Disease. The principal investigator of the study is Dr. Thacker Vinit Arvindbhai under the guidance of Dr. M.S.Khanpet

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 sample for the study.

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 you can later change my 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 sponsorer may stop 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.

Voluntary participation/ withdrawal: Your participation in this study is entirely voluntary and you may withdraw from the study at any time.

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 MD degree, review and publishing.

If you have any questions about my rights as participant you may call:

1) Dr. Thacker Vinit Arvindbhai
Investigator,
Postgraduate Student,
Department of Medicine,
JNMC , Belgaum.
Phone- 8722689139

2) Dr. M. S. Khanpet MD DNB(Nephrology)
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3)Dr. V. A. Kothiwale MD PhD
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4) Dr. P. V. Patil MD PhD DHA FIC(Path)
Professor and Head ,Chairman IEC,
Department of Pathology,
JNMC, Belgaum
Phone-0831-2473788,Extn-1527

CONSENT FORM

I voluntarily agree to take part in this study by signing on the line below. I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicated that I have read this entire consent form or it has been read to me, and has been explained to me in my vernacular language and had all my questions answered. I will be given a copy of this consent form.

Signature /Left Thumb print of the Participant or legally authorized representative.

Participant's Name/ Signature/ Left Thumb	: …….
Impression of the participant's	: …….
Name of the legally authorised representative/ Guardian	: …….
Signature/ Left Thumb Impression.	: …….
Witness's Name	: …….
Signature/ Left Thumb Impression.	:…….
Investigators name and Signature	: …….
Date and Place	: …….

Dr. M.S Khanpet MD, DNB(Nephrology)
Professor,
Dept. of Medicine, J. N. Medical College,
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Department of Medicine,
J.N. Medical College,
Belgaum
Ph. No. 0831-2473777
Ext. 1782

Annexures

Annexure II

ANNEXURE II – PROFORMA

Patient Name: I.P number:

Age: Sex:

Address:

Occupation:

Date of admission: Date of discharge:

SYMPTOMS :

1. Fever Yes/No
2. Cough with expectoration Yes/No
3. Breathlessness Yes/No
4. Burning micturition Yes/No
5. Headcahe Yes/No
6. Vomiting Yes/No
7. Altered consciousness Yes/No
8. Abdominal Pain Yes/No
9. Decreased urine output Yes/No

PAST HISTORY:

History of Hypertension
Duration of Diabetes
History of HIV infection

FAMILY HISTORY:

H/O Diabetes, Hypertension, Cardiovascular diseases

TREATMENT HISTORY:

Hemodialysis	Yes/No
Renal Transplant	Yes/No
Oral Hypoglcemics/ Insulin	
Use of Anti-hypertensives	Yes/No
Use of Statins	Yes/No

PERSONAL HISTORY:

H/o Smoking	Yes/No
H/o Alcohol consumption	Yes/No
Oral Contraceptive Pill use	Yes/No

PHYSICAL EXAMINATION:

ANTHROPOMETRY:

Weight

Height

Waist circumference

Abdominal Girth

Body Mass Index

GENERAL PHYSICAL EXAMINATION:

Pallor:	Yes/No
Icterus:	Yes/No
Lymphadenopathy:	Yes/No
Cyanosis:	Yes/No
Clubbing:	Yes/No
Edema:	Yes/No

VITALS:

Temperature:

Pulse:

Respiratory rate:

Blood pressure:

SYSTEMIC EXAMINATION:

Respiratory System

Cardiovascular System

Central Nervous System

Per Abdomen

LABORATORY INVESTIGATIONS

- Random Blood Sugar
- Glycosylated Hemoglobin
- Serum Creatinine
- Blood Urea
- Total Leucocyte count
- C-Reactive Protein
- Erythrocyte Sedimentation Rate
- Serum Triglyceride
- Serum Adionectin (ELISA)
- Estimated Glomerular Filtration Rate

ANNEXURE III - MASTER CHART

Serial Number	In patient number	Age (Years)	Sex	History				Symptoms				Physical Examination										Systemic Examination				Investigations						
																		Vitals														
																				BP											
				Duration of DM(Years)	Hypertension	Family history of DKD	Diabetic Treatment	Breathlessness	Nausea/Voiting	Abdominal Pain	Decreased Urine output	Weight (Kgs)	Height (Cms)	Body mass index (Kg/m2)	Pallor	Edema	Diabetic Retinopathy	Pulse (bpm)	Respiratory Rate(cpm)	Systolic (mm Hg)	Diastolic (mm Hg)	Cardiovascular	Respiratory	Central Nervous system	Per Abdomen	RBS (mg/dL)	HbA1c (%)	Serum Creatinine (mg/dL)	C-reactiove protein	Proteinuria	eGFR	Adiponectin (µg/mL)
1	507107	73	M	12	Y	N	O	Y	Y	N	Y	67	170	23.18	+	-	+	110	43	150	90	N	N	N	N	234	6.8	1.8	-	-	39.51	12.6
2	507874	59	M	7	N	N	O	N	N	N	N	56	168	19.84	-	-	+	76	23	130	80	N	N	N	N	321	6.9	1.23	-	-	64.01	9.2
3	508082	64	F	10	N	Y	O	N	N	N	N	48	152	20.78	-	-	+	78	24	154	98	N	N	N	N	352	9.7	1.21	-	Tr	47.61	10.3
4	508080	54	M	5	Y	N	O	N	N	N	N	80	178	25.25	-	-	-	84	25	146	92	N	N	N	N	213	10.0	0.92	+	Tr	90.00	13.7
5	507203	71	M	20	Y	N	I	N	Y	N	N	76	173	25.39	+	+	+	87	21	140	90	N	N	N	N	245	9.9	1.37	-	-	54.40	13.0
6	507254	84	M	15	N	N	O	N	N	N	N	52	168	18.42	-	+	+	89	22	154	96	N	N	N	N	234	7.7	1.67	-	++	41.87	7.8
7	508261	60	F	8	Y	N	O	N	N	N	N	62	163	23.34	+	-	+	88	25	150	86	N	N	N	N	265	6.8	0.8	-	+	80.07	7.0
8	517587	61	M	9	N	N	O	N	N	N	Y	80	173	26.73	+	-	+	86	25	130	80	N	N	N	N	276	7.0	1.44	-	+	53.01	6.9
9	518305	53	M	5	N	Y	O	N	N	N	N	68	163	25.59	-	-	-	6	26	134	86	N	N	N	N	243	8.6	1.58	-	+	49.00	11.8
10	519780	52	M	8	N	N	O	N	N	N	N	76	178	33.99	-	+	+	89	23	142	88	N	N	N	N	218	7.1	1.1	-	++	74.71	9.9
11	519620	56	M	7	N	Y	O	N	N	N	Y	78	168	27.64	-	-	-	90	22	170	90	N	N	N	N	240	7.6	3.71	+	+++	18.10	8.4
12	517562	58	M	3	N	Y	I	N	N	Y	N	72	165	26.45	-	-	-	76	24	136	96	N	N	N	N	268	9.3	1.02	+	-	79.73	7.8
13	518176	91	M	21	N	Y	O	N	N	N	N	70	175	22.86	+	-	+	74	25	130	80	N	N	N	N	309	8.6	1.37	-	+	51.77	9.0
14	517395	58	M	6	N	N	I	N	Y	N	N	86	173	28.73	+	+	-	86	23	120	86	N	N	N	N	267	9.0	1.75	-	+	42.76	7.9
15	517357	70	F	13	N	Y	I	N	Y	N	Y	68	165	24.98	+	-	-	84	22	156	88	N	N	N	N	304	11.2	1.86	-	Tr	28.47	17.6
16	517122	80	F	18	Y	Y	I	N	Y	N	Y	70	157	28.4	-	+	+	88	24	170	100	N	N	N	N	243	10.4	1.58	-	+	33.45	9.8
17	516829	59	M	7	N	N	O	N	Y	Y	N	76	168	26.93	-	+	+	82	26	164	84	N	N	N	N	254	7.2	1.68	-	-	44.67	14.0
18	516442	64	M	6	N	Y	I	N	Y	N	N	72	170	24.91	-	-	-	76	21	154	90	N	N	N	N	267	9.3	1.58	-	Tr	47.16	13.2
19	516840	61	F	10	N	Y	I	N	Y	Y	N	80	168	28.34	-	-	+	86	24	166	94	N	N	N	N	165	9.4	2.23	+	+	23.74	18.6
20	516825	65	F	5	Y	Y	O	N	Y	N	Y	84	157	34.08	-	-	-	78	23	180	94	N	N	N	N	198	8.5	3.06	-	+++	16.27	19.0
21	514675	65	F	6	Y	Y	O	Y	N	N	Y	60	152	25.97	+	-	-	86	44	170	90	N	N	N	N	254	7.9	2.81	+	+	17.95	7.2
22	514474	55	M	3	N	N	O	N	N	N	N	78	178	24.62	+	-	-	85	25	130	70	N	N	N	N	154	6.9	1.4	-	+	55.92	7.4

ANNEXURE III - MASTER CHART

Serial Number	In patient number	Age (Years)	Sex	History				Symptoms				Physical Examination										Systemic Examination				Investigations						
																		Vitals														
																				BP											
				Duration of DM(Years)	Hypertension	Family history of DKD	Diabetic Treatment	Breathlessness	Nausea/Voiting	Abdominal Pain	Decreased Urine output	Weight (Kgs)	Height (Cms)	Body mass index (Kg/m2)	Pallor	Edema	Diabetic Retinopathy	Pulse (bpm)	Respiratory Rate(cpm)	Systolic (mm Hg)	Diastolic (mm Hg)	Cardiovascular	Respiratory	Central Nervous system	Per Abdomen	RBS (mg/dL)	HbA1c (%)	Serum Creatinine (mg/dL)	C-reactiove protein	Proteinuria	eGFR	Adiponectin (µg/mL)
23	514645	60	F	9	N	N	O	N	N	N	Y	50	152	21.64	+	-	+	84	26	126	80	N	N	N	N	367	6.7	3.09	-	+++	16.35	11.7
24	515680	43	M	2	N	N	O	N	N	N	Y	80	175	26.12	+	-	-	88	23	140	90	N	N	N	N	345	7.4	2.55	-	++	29.43	13.1
25	512877	71	F	5	N	Y	I	N	N	N	Y	50	152	21.64	-	+	-	78	24	130	90	N	N	N	N	147	8.8	2.22	+	++	23.14	8.2
26	513524	65	M	4	N	Y	O	Y	N	N	Y	48	155	19.98	-	+	-	90	48	124	86	N	N	N	N	134	6.4	3.54	-	++	18.53	10.2
27	512694	59	M	6	N	N	I	N	N	N	N	70	180	21.6	+	+	-	93	22	118	78	N	N	N	N	254	9.3	1.21	-	-	63.01	6.8
28	513827	72	F	15	N	Y	I	N	N	N	Y	52	147	24.06	+	-	+	87	21	140	90	N	N	N	N	309	12.6	3.02	-	-	16.27	7.9
29	513864	61	F	6	Y	Y	O	N	N	N	N	56	153	23.92	-	-	-	92	24	134	86	N	N	N	N	165	6.3	1.21	-	+	48.08	10.9
30	524799	60	F	4	N	N	O	N	N	N	N	55	163	20.7	-	-	-	82	21	144	92	N	N	N	N	198	8.1	1.1	-	Tr	53.85	11.7
31	524297	66	F	8	N	N	I	N	N	N	N	56	160	21.87	+	+	+	75	22	142	92	N	N	N	N	206	9.7	1.1	-	-	52.82	18.3
32	523669	56	F	5	N	N	O	N	N	N	N	60	157	24.34	+	-	-	94	22	146	90	N	N	N	N	176	6.4	1.3	-	-	45.03	13.8
33	524316	43	M	4	N	N	I	N	N	N	N	65	175	21.22	+	-	-	75	21	136	88	N	N	N	N	205	9.0	1.23	-	Tr	68.26	13.1
34	524296	45	M	3	N	Y	I	N	N	N	N	85	183	25.38	+	+	-	86	24	142	96	N	N	N	N	214	9.3	2.4	+	-	31.27	14.7
35	524518	55	M	6	Y	Y	I	N	N	N	N	65	168	23.03	-	-	+	88	24	138	90	N	N	N	N	265	11.1	1.78	-	-	42.39	12.6
36	541088	55	F	8	N	Y	I	N	N	N	N	65	157	26.37	+	+	+	82	25	134	88	N	N	N	N	387	14.0	1.52	-	Tr	37.74	9.6
37	538321	70	F	9	N	Y	O	N	N	N	Y	60	152	25.97	+	+	+	86	26	150	90	N	N	N	N	202	7.6	2.1	-	Tr	24.75	8.2
38	538219	42	M	5	Y	Y	I	N	N	N	N	55	170	19.03	-	-	-	88	23	144	86	N	N	N	N	406	18.1	1.7	-	Tr	47.21	10.9
39	536850	74	M	17	N	N	I	N	N	N	N	72	167	25.82	+	-	+	90	26	136	86	N	N	N	N	254	11.8	1.77	-	+	40.17	11.7
40	539756	68	F	13	Y	Y	I	N	N	N	Y	48	142	23.8	+	+	+	94	24	128	78	N	N	N	N	256	12.0	1.57	+	+	34.82	6.9
41	544868	75	M	10	N	N	O	N	N	N	N	63	165	23.14	-	-	+	98	21	120	86	N	N	N	N	198	7.6	0.9	-	Tr	87.43	9.0
42	516260	69	M	8	N	N	O	N	N	N	N	80	155	33.3	+	+	-	100	23	134	88	N	N	N	N	167	7.3	1.76	-	+	41.01	12.4
43	516137	62	M	12	N	Y	I	N	N	N	N	78	171	26.67	+	-	+	86	24	140	90	N	N	N	N	206	10.1	2.01	+	+	35.96	12.6

ANNEXURE III - MASTER CHART

Serial Number	In patient number	Age (Years)	Sex	History				Symptoms				Physical Examination										Systemic Examination				Investigations						
																		Vitals														
																				BP											
				Duration of DM(Years)	Hypertension	Family history of DKD	Diabetic Treatment	Breathlessness	Nausea/Voiting	Abdominal Pain	Decreased Urine output	Weight (Kgs)	Height (Cms)	Body mass index (Kg/m2)	Pallor	Edema	Diabetic Retinopathy	Pulse (bpm)	Respiratory Rate(cpm)	Systolic (mm Hg)	Diastolic (mm Hg)	Cardiovascular	Respiratory	Central Nervous system	Per Abdomen	RBS (mg/dL)	HbA1c (%)	Serum Creatinine (mg/dL)	C-reactiove protein	Proteinuria	eGFR	Adiponectin (µg/mL)
44	516270	70	M	16	N	Y	I	N	N	N	Y	78	163	29.36	+	+	+	84	23	146	88	N	N	N	N	293	9.5	2.62	-	++	25.84	9.6
45	516055	75	M	15	N	Y	O	N	N	N	Y	70	157	28.4	+	+	+	76	25	156	98	N	N	N	N	341	8.7	2.09	-	++	33.07	11.6
46	536041	65	M	9	N	N	O	N	N	N	N	74	162	28.2	-	-	+	78	21	130	70	N	N	N	N	193	7.9	1.15	-	Tr	67.83	12.4
47	532316	69	M	14	N	N	I	N	N	N	N	71	157	28.8	-	-	+	82	23	136	86	N	N	N	N	271	9.8	1.21	-	+	63.20	12.7
48	531866	45	F	4	N	N	O	N	N	N	N	48	148	21.91	-	-	-	84	23	140	90	N	N	N	N	192	8.0	1.39	-	Tr	43.58	10.2
49	531592	58	F	8	Y	N	I	N	N	N	N	56	147	25.92	-	-	-	98	21	130	70	N	N	N	N	260	14.2	0.97	-	-	62.69	13.2
50	542682	57	M	7	N	N	O	N	N	N	N	78	168	27.64	-	-	-	96	22	132	82	N	N	N	N	173	6.9	1.07	-	-	75.71	11.7
51	525969	57	F	6	Y	N	O	N	N	N	N	50	143	24.45	+	-	+	94	21	138	92	N	N	N	N	183	7.4	0.86	-	++	72.29	15.1
52	543924	61	M	8	N	N	O	N	N	N	N	68	175	22.2	+	+	+	86	23	120	80	N	N	N	N	179	7.2	0.96	-	-	84.63	13.7
53	542069	63	F	10	Y	N	O	N	N	N	N	54	157	21.91	-	-	+	84	24	134	78	N	N	N	N	238	8.5	0.84	-	-	72.78	14.2
54	540662	65	M	11	N	N	I	N	N	N	N	65	167	23.31	-	-	-	86	23	120	80	N	N	N	N	249	9.6	0.95	-	-	84.57	12.7
55	528413	65	M	9	N	N	I	N	N	N	N	79	168	27.99	+	-	+	98	23	130	90	N	N	N	N	284	9.1	1.1	+	Tr	71.40	11.5
56	527621	64	M	6	N	N	I	N	N	N	N	86	170	29.76	-	-	-	110	22	136	92	N	N	N	N	274	10.8	1	-	+	79.96	11.8
57	525436	65	M	13	Y	N	O	N	N	Y	N	73	168	25.86	+	+	+	98	21	140	90	N	N	N	N	261	6.7	1.48	+	-	50.70	7.5
58	512878	52	F	4	Y	N	I	N	N	N	N	47	152	20.34	+	+	-	86	23	136	90	N	N	N	N	279	9.2	1.36	-	+	43.40	9.6
59	526072	72	M	16	N	N	O	N	Y	N	N	56	148	25.57	-	-	+	84	24	134	86	N	N	N	N	161	6.8	1.3	-	+	57.67	7.9
60	538612	53	M	3	N	N	I	N	N	N	N	67	162	25.53	-	-	-	76	23	130	80	N	N	N	N	298	14.0	1.19	-	++	67.97	9.0
61	539455	59	M	4	N	N	I	N	N	N	N	66	172	22.31	-	-	-	74	24	126	78	N	N	N	N	345	13.4	0.94	-	-	87.31	13.5
62	536137	78	F	10	N	Y	O	N	Y	N	N	50	152	21.64	+	-	+	84	25	124	74	N	N	N	N	256	7.4	1	+	Tr	56.99	10.0
63	508937	63	M	7	Y	Y	I	N	Y	N	N	76	168	26.93	+	-	+	78	22	146	88	N	N	N	N	268	9.0	1.37	-	++	55.78	7.7
64	510583	81	F	14	N	Y	O	N	Y	N	Y	45	144	21.7	+	+	+	82	23	160	80	N	N	N	N	344	7.3	1.98	-	-	25.71	7.2

ANNEXURE III - MASTER CHART

Serial Number	In patient number	Age (Years)	Sex	History				Symptoms				Physical Examination										Systemic Examination				Investigations						
																		Vitals														
																				BP											
				Duration of DM(Years)	Hypertension	Family history of DKD	Diabetic Treatment	Breathlessness	Nausea/Voiting	Abdominal Pain	Decreased Urine output	Weight (Kgs)	Height (Cms)	Body mass index (Kg/m2)	Pallor	Edema	Diabetic Retinopathy	Pulse (bpm)	Respiratory Rate(cpm)	Systolic (mm Hg)	Diastolic (mm Hg)	Cardiovascular	Respiratory	Central Nervous system	Per Abdomen	RBS (mg/dL)	HbA1c (%)	Serum Creatinine (mg/dL)	C-reactiove protein	Proteinuria	eGFR	Adiponectin (µg/mL)
65	499035	60	F	9	N	N	I	N	N	N	N	78	150	34.67	+	-	-	76	24	128	88	N	N	N	N	387	10.9	0.92	-	-	66.18	10.1
66	499076	69	M	12	N	Y	O	N	N	N	N	86	162	32.77	+	-	+	86	26	132	88	N	N	N	N	154	8.0	1.4	-	-	53.41	7.4
67	507271	63	M	10	N	Y	I	Y	Y	Y	Y	66	156	27.12	-	-	+	84	45	146	86	N	N	N	N	179	10.7	2.9	-	++	23.48	6.2
68	505021	73	F	14	Y	N	I	N	N	N	N	54	148	24.65	-	-	+	92	22	148	96	N	N	N	N	212	10.1	0.94	-	+	62.04	13.5
69	506889	56	F	6	N	N	O	N	N	N	N	75	150	33.33	-	-	-	86	24	134	84	N	N	N	N	166	7.4	0.9	-	Tr	68.84	13.0
70	506779	56	M	5	N	N	O	N	N	N	N	74	168	26.22	-	-	-	86	22	120	80	N	N	N	N	238	8.0	0.95	-	Tr	87.16	12.2
71	506610	54	F	3	N	N	O	N	N	N	N	66	143	32.28	+	-	+	92	23	126	76	N	N	N	N	279	7.4	0.81	-	+	78.32	14.8
72	506519	48	M	5	N	N	I	N	N	N	N	90	176	29.05	+	-	-	84	21	130	82	N	N	N	N	367	12.8	0.97	-	-	87.80	11.0
73	506041	69	M	14	N	N	I	N	N	N	N	88	158	35.25	+	+	+	98	24	140	90	N	N	N	N	307	9.0	1.3	+	-	58.17	8.1
74	506641	54	F	6	Y	N	I	N	N	N	N	76	154	32.05	+	-	+	86	22	148	96	N	N	N	N	285	15.1	0.97	-	+	63.61	12.4
75	522160	54	M	8	N	N	O	N	N	N	N	82	165	30.12	+	-	+	84	23	126	84	N	N	N	N	258	6.9	1.27	-	Tr	62.81	11.0
76	520823	64	F	10	N	N	O	N	N	N	N	56	153	23.92	+	-	+	86	21	136	84	N	N	N	N	284	8.5	0.9	-	Tr	67.00	13.9
77	521488	59	M	11	Y	N	I	N	N	N	N	73	160	28.52	+	-	+	82	23	156	98	N	N	N	N	189	9.4	1.14	-	-	69.88	11.4
78	519326	59	M	8	N	N	I	N	N	Y	N	86	171	29.41	+	-	+	92	21	138	92	N	N	N	N	262	11.6	1.62	+	-	46.59	6.9
79	509407	49	M	12	N	N	I	N	Y	N	Y	80	168	28.34	+	-	+	90	23	124	84	N	N	N	N	274	11.6	2.28	-	++	32.61	6.7
80	514097	49	M	4	Y	Y	I	N	N	N	N	79	165	29.02	-	-	-	76	23	142	94	N	N	N	N	259	9.9	0.97	-	+	87.43	11.2
81	530387	50	M	6	Y	N	O	N	N	N	N	73	167	26.18	-	-	-	96	24	146	98	N	N	N	N	260	8.3	1	-	-	84.07	10.9
82	531855	73	F	16	N	N	O	N	N	N	Y	69	148	31.5	-	+	+	72	21	126	78	N	N	N	N	247	8.9	1.4	-	-	39.18	8.1
83	535493	79	F	20	N	Y	O	N	N	N	N	75	150	33.33	-	-	+	74	25	120	80	N	N	N	N	149	6.7	0.88	-	Tr	65.88	10.0
84	534022	58	M	13	Y	Y	O	N	N	N	N	69	158	27.64	-	-	+	76	24	134	86	N	N	N	N	292	8.3	1.13	-	-	70.84	9.9
85	534579	59	M	10	N	N	I	N	N	N	N	73	168	25.86	-	-	+	86	25	134	82	N	N	N	N	387	11.6	0.99	-	-	82.24	11.9

ANNEXURE III - MASTER CHART

Serial Number	In patient number	Age (Years)	Sex	History				Symptoms				Physical Examination										Systemic Examination				Investigations						
																		Vitals														
																				BP											
				Duration of DM(Years)	Hypertension	Family history of DKD	Diabetic Treatment	Breathlessness	Nausea/Voiting	Abdominal Pain	Decreased Urine output	Weight (Kgs)	Height (Cms)	Body mass index (Kg/m2)	Pallor	Edema	Diabetic Retinopathy	Pulse (bpm)	Respiratory Rate(cpm)	Systolic (mm Hg)	Diastolic (mm Hg)	Cardiovascular	Respiratory	Central Nervous system	Per Abdomen	RBS (mg/dL)	HbA1c (%)	Serum Creatinine (mg/dL)	C-reactiove protein	Proteinuria	eGFR	Adiponectin (µg/mL)
86	531609	52	M	7	N	Y	O	N	N	N	N	87	173	29.07	+	-	-	94	22	124	88	N	N	N	N	261	8.9	1.14	-	Tr	71.70	10.7
87	535805	70	M	19	Y	Y	O	N	N	N	N	72	168	25.51	+	-	+	84	23	146	96	N	N	N	N	184	6.9	1.3	+	-	58.01	7.8
88	530727	43	F	4	N	N	I	N	N	N	N	80	158	32.05	-	-	-	86	24	136	78	N	N	N	N	295	13.9	0.93	-	-	69.93	12.7
89	531194	62	M	12	N	N	I	N	N	N	N	84	171	28.73	-	-	+	82	23	134	84	N	N	N	N	284	10.7	1.23	-	Tr	63.37	10.2
90	527402	78	F	15	Y	N	O	N	N	N	N	71	154	19.94	+	-	+	92	22	158	96	N	N	N	N	205	6.9	0.91	-	Tr	63.55	12.6
91	522823	81	M	23	Y	Y	I	N	N	N	N	80	168	28.34	+	-	+	88	21	172	94	N	N	N	N	399	10.5	1.2	-	+	61.76	10.0
92	523572	62	F	14	N	Y	O	N	N	N	N	68	143	33.25	+	-	+	84	23	138	84	N	N	N	N	376	7.5	1.13	+	-	51.86	9.0
93	535180	52	M	9	N	N	I	N	N	N	N	93	170	32.18	-	-	-	76	21	140	88	N	N	N	N	394	15.4	1.2	-	-	67.58	12.8
94	538291	59	M	8	N	Y	I	N	N	N	N	87	167	31.2	+	-	-	72	22	136	84	N	N	N	N	479	15.3	1.12	-	-	71.32	13.0
95	539244	64	F	12	N	N	O	N	N	N	N	65	150	28.89	-	-	+	74	24	124	84	N	N	N	N	247	6.7	0.71	-	-	88.09	15.2
96	536244	74	M	20	N	Y	O	N	N	Y	N	74	166	26.85	+	-	+	78	25	134	84	N	N	N	N	367	8.7	1.3	+	+	57.35	7.6
97	531599	41	F	4	N	Y	I	N	N	N	N	63	148	28.76	-	-	-	86	22	126	82	N	N	N	N	298	12.5	0.89	-	-	74.29	13.0
98	533398	53	F	8	Y	Y	I	N	N	N	N	67	165	24.61	-	-	+	94	25	148	86	N	N	N	N	342	9.0	0.93	-	Tr	67.03	10.8
99	511164	66	M	14	N	N	O	N	N	N	N	97	171	33.17	+	-	+	92	26	130	82	N	N	N	N	219	8.7	1.3	+	+	58.70	7.9
100	533372	81	F	17	N	N	O	N	N	N	N	69	154	29.09	+	+	+	86	24	138	86	N	N	N	N	269	7.5	1.2	+	+	45.83	8.3

Annexures

Annexure III

ANNEXURE III – KEY TO MASTER CHART

-	-	Negative
+	-	Positive
µg/mL	-	Micro gram per milli litre
BP	-	Blood pressure
bpm	-	Beats per minutes
Cms	-	Centimeters
DKD	-	Diabetic kidney disease
DM	-	Diabetes mellitus
eGFR	-	Estimated glomerular filtration rate
F	-	Female
HbA1c	-	Glycated haemoglobin
I	-	Insulin
Kg/m^2	-	Kilogram per square meter
Kgs	-	Kilograms
M	-	Male
mg/dL	-	Milligram per deciliter
mm Hg	-	Millimeter of mercury
N	-	No
O	-	Oral
Tr	-	Traces
Y	-	Yes