“ESTIMATION OF LEVELS OF VITAMIN D IN TYPE 2 DIABETES MELLITUS - ONE YEAR CROSS-SECTIONAL STUDY”

REG NO. BG0111001

Dissertation

Submitted to the
KLE University, Belgaum, Karnataka

In Partial Fulfillment
of the requirements for the degree of

M. D.
in
GENERAL MEDICINE

DEPARTMENT OF MEDICINE,
JAWAHARLAL NEHRU MEDICAL COLLEGE,
BELGAUM, KARNATAKA

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ENDORSEMENT

This is to certify that the dissertation entitled **"ESTIMATION OF LEVELS OF VITAMIN D IN TYPE 2 DIABETES MELLITUS - ONE YEAR CROSS-SECTIONAL STUDY"** is a bonafide research work done by THE CANDIDATE **REG NO. BG0111001**.

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LIST OF ABBREVIATIONS USED

α	-	Alpha
β	-	Beta
µg	-	Micro gram
1,25(OH)2D3	-	1α, 25-dihydroxyvitamin D3
ADA	-	American Diabetes Association
Akt	-	Protein kinase B
BMI	-	Body mass index
CDC	-	Center for Disease Control
CoA	-	Co-activator
CoR	-	Co-repressor
CV	-	Co-efficient of variation
DBP	-	Diastolic blood pressure
DCCT	-	Diabetes Control and Complications Trial
DM	-	Diabetes mellitus
DNA	-	Deoxyribonucleic acid
e.g.	-	For example
ESRD	-	End stage renal disease
FPG	-	Fasting plasma glucose
GAD	-	Glutamic acid decarboxylase
GDM	-	Gestational diabetes mellitus
GLUT	-	Glucose transporter
HbA1c	-	Glycated hemoglobin
HDL	-	High density lipoprotein
HLA	-	Human leukocyte antigen

HNF	-	Hepatocyte nuclear transcription factor
HOMA-IR	-	Homoeostatic model assessment for Insulin resistance
hrs	-	Hours
ICMR	-	Indian Council of Medical Research
IDDM	-	Insulin dependent diabetes mellitus
IDF	-	International Diabetes Federation
IFG	-	Impaired fasting glycaemia
IGF	-	Impaired fasting glucose
IGT	-	Impaired glucose tolerance
IPF	-	Insulin promoter factor
IU	-	International units
Kg	-	Kilogram
LDL	-	Low density lipoprotein
m	-	Meter
mg/dl	-	Milligram per deci litre
min	-	Minute
mm Hg	-	Millimeter of mercury
MODY	-	Maturity Onset Diabetes of Young
n	-	Total number
NIDDM	-	Non-insulin dependent diabetes mellitus
NK	-	Natural killer
nmol	-	Nano mole
nmols/L	-	Nano moles per litre
NPDR	-	Non Proliferative Diabetic Retinopathy
NSGP	-	National Glycohemoglobin Standardization Program

OGTT	-	Oral glucose tolerance test
p	-	Prevalence
POL II	-	Polymerase II
PPBS	-	Post prandial blood sugar
RNA	-	Ribonucleic acid
RR	-	Relative risk
RXR	-	Retinoic X receptor
SD	-	standard deviation
SGOT	-	Serum glutamic oxaloacetic transaminase
SGPT	-	Serum glutamic-pyruvic transaminase
STZ	-	Streptozotocin
Tc	-	Cytotoxic
TCF7L2	-	Transcription factor 7–like 2
TSH	-	Thyroid stimulating hormone
VDR	-	Vitamin D response
VDRE	-	Vitamin D response element
WHO	-	World health organization

ABSTRACT

Background and objectives

Recently, Vitamin D has sparked widespread interest in the pathogenesis and prevention of diabetes. The present study was undertaken to estimate the levels of Vitamin D in patients with Type 2 Diabetes Mellitus and to correlate levels of Vitamin D with Glycemic status of Diabetes Mellitus.

Methodology

The present one year cross sectional study was carried out in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum from January 2012 to December 2012. A total of 100 patients with type 2 diabetes mellitus were studied. The estimation of Vitamin D was done using Siemens, ADVIA Centraur VitD assay, a one pass 18 minute antibody competitive immunoassay.

Results

Maximum number of cases were in the age group of 51 to 60 that is 32 patients (32%). The mean age of study population was 60.6 ± 11.28 years. Out of 100 patients, 62 (62%) were males and 38 patients (38%) were females, with a ratio of male to female 1.63:1. In more than 87 patients (87%), the levels of Vitamin-D were below normal, in 7 patients (7%) the levels were insufficient and in remaining 6 patients (6%) the levels were either normal or more than normal. Most of the patients had fasting and post prandial glucose abnormality (98% each) and HOMA-IR was >3.8 in 60 patients (60%).

Conclusion and interpretation

The results observed were a significant correlation of Vitamin-D levels with variable factors – post-prandial blood sugar, HbA1c, HOMA-IR, cholesterol and low density lipoprotein (LDL). However, we did not find significant correlation with variable factors – age, gender, duration, body mass index, fasting blood sugar, high density lipoprotein (HDL) and triglycerides.

Keywords

Diabetes mellitus; Glycaemic control; Insulin resistance; Vitamin D;

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Chapter 1

Introduction

INTRODUCTION

Diabetes mellitus (DM) is one of the oldest diseases known to man. It was first reported in Egyptian manuscript about 3000 years ago.[1] Diabetes can be distinguished into two types, Type 1 and Type 2. In 1936, the distinction between Type 1 and Type 2 DM was made clearly.[2] Type 2 DM was first described as a component of metabolic syndrome in 1988.[3]

Type 1 Diabetes is a form involving little or no production of insulin by the pancreas. It has often an early onset and involves medication and monitoring of blood-sugar levels. Type 2 Diabetes is characterized by problems with insulin-receptors. It has often a late onset, and is associated with reduced physical activity and overweight. Medication and monitoring of blood-sugar levels are often necessary.[2]

T2DM is a non-autoimmune, complex, heterogeneous and polygenic metabolic disease condition in which the body fails to produce enough insulin, characterized by abnormal glucose homeostasis. Its pathogenesis appears to involve complex interactions between genetic and environmental factors.[4] T2DM occurs when impaired insulin effectiveness (insulin resistance) is accompanied by the failure to produce sufficient cell insulin.[5]

It is estimated that 366 million people had DM in 2011; by 2030 this would rise to 552 million. The number of people with Type 2 DM is increasing in every country with 80% of people with DM living in low- and middle-income countries. DM caused 4.6 million deaths in 2011.[6]

The incidence of Type 2 DM varies substantially from one geographical region to the other as a result of environmental and lifestyle risk factors.[7] Thirty years ago, the prevalence of diabetes in India based on the Indian Council of Medical Research (ICMR) multicentric survey[8] was around 2% in urban India and 1% in rural India. In just three decades, these prevalence rates have shot up to 12 to 16% in urban India and 3 to 8% in rural India, in adults over 20 years of age. This represents a 600 to 800% increase in prevalence rates of diabetes something which is unparallel in any Western nation. Indeed, India is now referred as the "Diabetic Capital" of the world.

It is predicted that the prevalence of DM in adults of which Type 2 DM is becoming prominent will increase in the next two decades and much of the increase will occur in developing countries where the majority of patients are aged between 45 and 64 years.[9]

Insulin plays a crucial role in this disease. Insulin is a hormone produced by the pancreas. This hormone regulates carbohydrates and fat metabolism. Glycated hemoglobin (or HbA1c) can be measured in the blood to find out the amount of sugar in the blood.[10]

Further, Type 2 DM is associated with several complications. The complications of Type 2 diabetes mellitus include retinopathy, nephropathy, and neuropathy (both peripheral and autonomic). The risk for atherosclerotic vascular disease is also increased in patients with Type 2 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.[2]

Although important knowledge has been acquired on the etiology of diabetes its precise etiopathogenesis is still under discussion. Inflammatory factors, reactive oxygen species and autoimmune reactions have all strongly emerged as the major pathogenic effectors for diabetes.

Recently, Vitamin D has sparked widespread interest in the pathogenesis and prevention of diabetes. Vitamin D is a steroid vitamin which promotes the intestinal absorption and metabolism of calcium and phosphorus. There are several terms used to describe Vitamin D. Vitamin D2 or ergocalciferol is synthesized by plants. Vitamin D3 or cholecalciferol is synthesized in the human skin when it has been exposed to ultraviolet rays from sunlight. Food may contain Vitamin D2 or D3. Serum 25-hydroxyvitamin D level is the best marker of whole-body Vitamin D status. Biologically active form of Vitamin D is 1,25-dihydroxy vitamin D.[11]

It is postulated that, Vitamin D as the major regulator for calcium homeostasis, it directly and or indirectly improves insulin exocytosis via activating calcium-dependent endopeptidases. Vitamin D also improves glucose tolerance.[12] Vitamin D could also prevent type 2 diabetes mellitus through its role as an efficient antioxidant. Additionally, the steroid hormone form of Vitamin D promotes suppressor cell activity and inhibits the generation of cytotoxic (Tc), macrophages, delayed hypersensitivity type and natural killer (NK) cells.[13] Vitamin D also mediates several non-calcemic functions. It is a regulator of

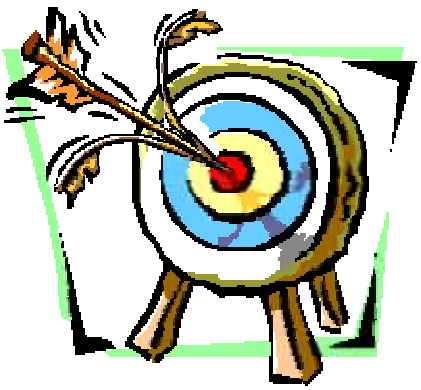
cellular proliferation, differentiation and replication, and mediator of autoimmune reactions, in a variety of organs and biological systems. The discovery of receptors for 1α, 25-dihydroxyvitamin D3 (1,25(OH)2D3), the activated form of Vitamin D, in tissues with no direct role in calcium and bone metabolism (e.g., pancreatic beta-cells and cells of the immune system) has broadened our view of the physiological role of Vitamin D.[14,15]

This novel risk factor in the development of diabetes has recently gained attention and remains unexplored.[16] The study from Vacek et al. (2012) found an important role of Vitamin D in cardiovascular health and diabetes, and found that Vitamin D deficiency was related to reduced survival.[17] The role of Vitamin D in type 2 diabetes mellitus is suggested by cross-sectional studies showing that low serum concentrations of 25-hydroxyvitamin D are associated with impaired glucose tolerance and diabetes.[18,19] Vitamin D helps in insulin secretion by stimulating beta cell insulin secretion or it may facilitate the conversion of pro-insulin to insulin. Vitamin D improves insulin sensitivity and promote beta cell survival by modulating the effects of cytokines.[20] Therefore, Vitamin D deficiency may be implicated in pathogenesis of Type 2 Diabetes Mellitus.

However, the role of Vitamin D in type 2 diabetes mellitus remains unexplored. So far, very few studies have explored the role of Vitamin D and type 2 diabetes mellitus in India. Hence the present study was undertaken to estimate the levels of Vitamin D in patients with Type 2 Diabetes Mellitus and to correlate levels of Vitamin D with Glycemic status of Diabetes Mellitus.

Chapter 2

Objectives



OBJECTIVES

The objectives of the present study were :

- To estimate the levels of Vitamin D in patients with Type 2 Diabetes Mellitus.
- To correlate levels of Vitamin D with Glycemic status of Diabetes Mellitus.

Chapter 3

Review of Literature

REVIEW OF LITERATURE

Diabetes mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of diabetes mellitus exist and are caused by a complex interaction of genetics, environmental factors, and life style choices. Depending on the etiology of diabetes mellitus, factors contributing to hyperglycemia may include reduced insulin secretion, decreased glucose utilization, and increased glucose production. The metabolic dysregulation associated with diabetes mellitus causes secondary pathophysiologic changes in multiple organ systems. Diabetes mellitus is the leading cause of end-stage renal disease (ESRD), non-traumatic lower extremity amputation and adult blindness. With an increasing incidence worldwide, diabetes mellitus will be a leading cause of morbidity and mortality in the future.[1]

History of diabetes mellitus

Diabetes mellitus (DM) is probably one of the oldest diseases known to man. It was first reported in Egyptian manuscript about 3000 years ago.[1] In 1936, the distinction between Type 1 and Type 2 DM was made clearly. Type 2 DM was first described as a component of metabolic syndrome in 1988. Type 2 DM (formerly known as non-insulin dependent DM) is the most common form of DM characterized by hyperglycemia, insulin resistance, and relative insulin deficiency. Type 2 DM results from interaction between genetic, environmental and behavioral risk factors.[21]

Diabetes mellitus is a disease that was recognized in antiquity, but its history has been characterized by numerous cycles of discovery, neglect and

rediscovery. Its history may be divided into four major periods that reflect different phases in the understanding and management of the disease. The 'ancient' period witnessed the first clinical descriptions of diabetes and complications. The 16th to 18th centuries have been termed as the 'diagnostic' period, as diabetes mellitus was then identified as a separate disease entity, while the mid to late 19th century may be regarded as the first 'experimental' period, during which the glucoregulatory role of the pancreas became clear and the biochemical disturbances of diabetes were initially characterized.[14] Finally, the 20th century has seen a dramatic increase in knowledge about diabetes. The discovery of insulin in 1921-22 has had profound scientific, clinical and social consequences.

Some key developments in scientific and clinical understanding of diabetes may be summarized as follows:[22]

- Polyuric states, clinically resembling diabetes mellitus were described as early as 1550 BC in the ancient Egyptian papyrus discovered by George Ebers.
- The sweet taste of diabetic urine was noted in the 5th and 6th century AD by the Indian physicians (Sushruta and Charaka) 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.

- 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.
- 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.
- In 1889, Oskar Minkowski and Josef Von Mering observed that total pancreatectomy produced diabetes in dogs.
- In 1893, Edovard Laguesse named the 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.
- Various workers, including George Zuelzer (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.
- 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 pancreatectomised dogs and were first tested in a human diabetic (Leonard Thompson) in January, 1922.

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

A. The sequencing of insulin in 1955 by Frederick Sanger and elucidation of its three dimensional structure in 1969 by Dorothy Hodgkin.
B. The measurement of insulin concentration using the first radio immunoassay, by Solomon Berson and Rosalyn Yalow in 1959.
C. The isolation of proinsulin in 1967 by Donald Steiner's group.
D. Identification of specific insulin receptors by Pierre Freychet and colleagues in 1971, and
E. The sequencing of the insulin receptor in 1985.

Mile stones in the management of diabetes have included:

A. The development of long acting insulin preparations (isophane) in 1936 by Hans Christian Hagedorn and colleagues.
B. The testing of sulfonylureas in 1944 by Auguste Loubatieres.
C. First therapeutic use of a biguanide (phenformin) in 1957 by G. Ungar.
D. Introduction in the late 1970's of dry reagent test strips suitable for self monitoring of blood glucose, and
E. Definitive proof from the diabetes control and complications trial (DCCT) published in 1993, that strict glycemic control could slow or prevent the development of diabetic microvascular complications.[23]

Classification of diabetes and other categories of glucose regulation

Diabetes mellitus 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.[2,24]

Etiologic classification of diabetes mellitus

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 (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 DNA
8. 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, fibrocalculous pancreatopathy.

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.

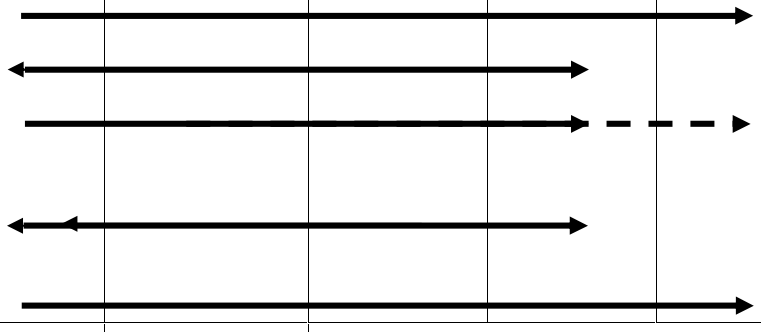
H. Other genetic syndromes sometimes associated with diabetes – Down's syndrome, Klinefelter's syndrome, Turner's syndrome, Wolfram's syndrome, Friedreich's ataxia, Huntington's chorea, Laurence-Moon-Biedl syndrome, myotonic dystrophy, porphyria, Prader-Willi syndrome.

IV. Gestational diabetes mellitus (GDM)

The two broad categories of diabetes mellitus are designated as type 1 and type 2. Type 1 results from autoimmune beta cell destruction, which leads to insulin deficiency. Type 2 diabetes mellitus is a heterogenous 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 diabetes mellitus.

Two features of the current classification of diabetes mellitus diverge from previous classifications. First, the terms ‘insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM) are obsolete. Since many individuals with Type 2 diabetes mellitus eventually require insulin treatment for control of hyperglycemia, the term NIDDM generates considerable confusion. Second, age is not a criterion in the classification system. Although type 1 diabetes mellitus most commonly develops before the age of 30, an autoimmune beta cell destructive process can develop at any age. It is estimated that between 5 to 10% of individuals who develop diabetes mellitus after age of 30 have type 1 diabetes mellitus. Like wise, type 2 diabetes mellitus more typically develops with increasing age, but it also occurs in children, particularly in obese adolescents.

Spectrum of glucose homeostasis and diabetes mellitus[2]

Type of diabetes	Normal glucose tolerance	Hyperglycemia			
		Impaired fasting glucose or impaired glucose tolerance	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)	< 110	110-125	≥ 126		
2-h pg (mg/dl)	< 140	140 – 199	≥ 200		

Type 1 diabetes mellitus

Immune Mediated Diabetes (Type 1A)

This form of diabetes, which accounts for only 5-10% of those with diabetes, results from a cellular mediated autoimmune destruction of the β-cells of pancreas. Markers of immune destruction of the β-cells include islet cell auto antibodies, autoantibodies to insulin, autoantibodies to glutamic acid decarboxylase (GAD65) and autoantibodies to the tyrosine phosphatases IA-2 and IA-2B. One and usually more of these auto antibodies are present in 85-90% of individuals when fasting hyperglycemia is initially recognized. Also the disease has strong HLA associations, with linkage to the DQA and DQB genes.

In this form of diabetes, the rate of β-cell destruction is quite variable. Being rapid in some individuals (mainly infants and children) and slow in others (mainly adults). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and / or ketoacidosis in the presence of infection or other stress. Immune mediated diabetes commonly occurs in childhood and adolescence, but it can occur at any age, even in 8^{th} and 9^{th} decade of life.

Usually associated with other autoimmune disorders such as - Grave's disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis and pernicious anemia.

Idiopathic Diabetes (Type 1B)

Some patients of type 1 diabetes mellitus have no known etiologies. These patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. This forms only a minority of patients with type 1 diabetes mellitus. Most patients are of African or Asian ancestry. Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes.

This form of diabetes is strongly inherited, lacks immunological evidence for β-cell autoimmunity, and is not HLA associated.

Type 2 diabetes mellitus

This form of diabetes, which accounts for ~90-95% of those with diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency.

There are probably many different causes of this form of diabetes. Specific etiologies are not identified. Most patients with type 2 diabetes mellitus are obese, and obesity itself causes some degree of insulin resistance. Ketoacidosis seldom occurs spontaneously. This form of diabetes frequently goes undiagnosed for many years, as the hyperglycemia develops gradually. Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications. The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity. It Occurs more frequently in women with prior GDM (Gestational diabetes mellitus), and individuals with hypertension or dyslipidemia. It is often associated with a strong genetic predisposition, more so than type 1 diabetes mellitus.

Other Specific Types of Diabetes Mellitus

Other etiologies of diabetes mellitus include specific genetic defects in insulin secretion or action, metabolic abnormalities that impair insulin secretion, mitochondrial abnormalities and a host of conditions that impair glucose tolerance. MODY (Maturity Onset Diabetes of Young) is a subtype of diabetes mellitus characterized by autosomal dominant inheritance, early onset of hyperglycemia, and impairment of insulin secretion.

Diabetes mellitus can result from pancreatic exocrine disease when the majority of pancreatic islets (>80%) are destroyed. Hormones that antagonize the action of insulin can lead to diabetes mellitus. Thus diabetes mellitus, is often a feature of endocrinopathies, such as acromegaly and Cushing's disease.

Gestational Diabetes Mellitus (GDM)

Glucose intolerance may develop during pregnancy. Insulin resistance related to the metabolic changes of late pregnancy increases insulin requirements and may lead to IGT. GDM occurs in approximately 4% of pregnancies. Most women revert back to normal glucose tolerance postpartum, but have a substantial risk (30%-60%) of developing diabetes mellitus later in life.[25]

Epidemiology

Worldwide

It is estimated that 366 million people had DM in 2011; by 2030 this would rise to 552 million. The number of people with Type 2 DM is increasing in every country with 80% of people with DM living in low- and middle-income countries. DM caused 4.6 million deaths in 2011.[21]

The incidence of Type 2 DM varies substantially from one geographical region to the other as a result of environmental and lifestyle risk factors.[7] According to The International Diabetes Federation,[26] the prevalence of diagnosed diabetes has more than doubled in the last three decades, largely because of the increase in obesity in United States. The top 10 countries in number of people with diabetes currently are India, China, the United States,

Indonesia, Japan, Pakistan, Russia, Brazil, Italy, and Bangladesh. The greatest percentage increase in rates of diabetes will occur in Africa over the next 20 years. Unfortunately, at least 80% of people in Africa with diabetes are undiagnosed, and many in their 30s to 60s will die from diabetes there.[27]

A 2011 Centers for Disease Control and Prevention (CDC) report estimated that nearly 26 million Americans have diabetes.[28] Additionally, an estimated 79 million Americans have prediabetes. Diabetes affects 8.3% of Americans of all ages, 11.3% of adults aged 20 years and older, and 25% of persons aged 65 and older, according to the National Diabetes Fact Sheet for 2011. About 27% of those with diabetes—7 million Americans—do not know that they have the disease. About 215,000 people younger than 20 years had diabetes (type 1 or type 2) in the United States in 2010.

Indian scenario

In the urban population, an Indian Council of Medical Research (ICMR) study in 1972 reported a prevalence of 2.3%[29] which rose to 12.1% in the year 2000.[30] More recently, Mohan et al. (2008a) provided estimates from a nationwide surveillance study of T2DM and found that in urban areas there was a prevalence of 7.3% of known T2DM and a prevalence of 3.2% in peri-urban/slum areas.

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.[31]

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.[32]

A study conducted by Zaman et al showed higher prevalence of diabetes (19.78%) in Karnataka[33] as well as Vijaykumar et al also showed higher prevalence of diabetes (12.5%) in Kerala, south India.[34]

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, appropriate intervention to increase physical activity and 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.[32]

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.[62]

Sex

Type 2 DM is slightly more common in older women than men.[62] Data from the NFHS of 2005-06 suggested that the number of women who have diabetes ranges from 0.28% women in Rajasthan to 2.54% women in Kerala. Five states (Tamil Nadu, Goa, Tripura, West Bengal, and Delhi) have relatively high (>1.5%) number of women with T2DM. Rajasthan, Uttar Pradesh, Assam, and Maharashtra have T2DM prevalence levels below 0.5%. Among men, six states (Kerala, Goa, Tripura, West Bengal, Andhra Pradesh, and Sikkim) have prevalence level >1.5%. Five states (Kashmir, Mizoram, Himachal Pradesh, Rajasthan, Uttar Pradesh) have prevalence below 0.5% for men.

A recent study conducted by Zaman et al showed the prevalence of diabetes was more in women (22.04%) compared to men (16.06%) in Karnataka people.[33]

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 obese individuals. In some areas, more Type 2 than Type 1 diabetes mellitus is being diagnosed in pre-pubertal children, teenagers, and young adults. Virtually all cases of diabetes mellitus in older individuals are Type 2.[35]

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 globalization of western pattern of lifestyle, along with poverty, lack of education, 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.[36]

Etiology

The etiology of type 2 diabetes mellitus appears to involve complex interactions between environmental and genetic factors. Presumably, the disease develops when a diabetogenic lifestyle (i.e., excessive caloric intake, inadequate caloric expenditure, obesity) is superimposed on a susceptible genotype.

The body mass index (BMI) at which excess weight increases risk for diabetes varies with different racial groups. For example, compared with persons of European ancestry, persons of Asian ancestry are at increased risk for diabetes at lower levels of overweight.[37] Hypertension and pre-hypertension are associated with a greater risk of developing diabetes in whites than in African Americans.[38]

In addition, an in-utero environment resulting in low birth weight may predispose some individuals to develop type 2 diabetes mellitus.[39-41] Infant

weight velocity has a small, indirect effect on adult insulin resistance, and this is primarily mediated through its effect on BMI and waist circumference.[42]

About 90% of patients who develop type 2 diabetes mellitus are obese. However, a large, population-based, prospective study has shown that an energy-dense diet may be a risk factor for the development of diabetes that is independent of baseline obesity.[43]

Some studies suggest that environmental pollutants may play a role in the development and progression of type 2 diabetes mellitus.[44] A structured and planned platform is needed to fully explore the diabetes-inducing potential of environmental pollutants.

Secondary diabetes may occur in patients taking glucocorticoids or when patients have conditions that antagonize the actions of insulin (e.g., Cushing syndrome, acromegaly, pheochromocytoma).

Major risk factors

The major risk factors for type 2 diabetes mellitus are the following:[45]

- Age greater than 45 years (though, as noted above, type 2 diabetes mellitus is occurring with increasing frequency in young individuals).
- Weight greater than 120% of desirable body weight.
- Family history of type 2 diabetes mellitus in a first-degree relative (e.g., parents or siblings).

- Hispanic, Native American, African American, Asian American, or Pacific Islander descent.

- History of previous impaired glucose tolerance (IGT) or impaired fasting glucose (IFG).

- Hypertension (>140/90 mm Hg) or dyslipidemia (HDL cholesterol level < 40 mg/dL or triglyceride level >150 mg/dL).

- Polycystic ovarian syndrome (which results in insulin resistance)

Genetic influences

The genetics of type 2 diabetes mellitus is complex and not completely understood. Evidence supports the involvement of multiple genes in pancreatic beta-cell failure and insulin resistance. Genome-wide association studies have identified dozens of common genetic variants associated with increased risk for type 2 diabetes mellitus.[46] Of the variants thus far discovered, the one with the strongest effect on susceptibility is the transcription factor 7–like 2 (*TCF7L2*) gene.

Identified genetic variants account for only about 10% of the heritable component of most type 2 diabetes.[48] An international research consortium found that use of a 40-SNP genetic risk score improves the ability to make an approximate 8-year risk prediction for diabetes beyond that which is achievable when only common clinical diabetes risk factors are used. Moreover, the predictive ability is better in younger persons (in whom early preventive strategies could delay diabetes onset) than in those older than 50 years.[47]

Depression

Accumulating evidence suggests that depression is a significant risk factor for developing type 2 diabetes. Pan et al found that the relative risk was 1.17 in women with depressed mood and 1.25 in women using antidepressants.[48] Antidepressant use may be a marker of more severe, chronic, or recurrent depression, or antidepressant use itself may increase diabetes risk, possibly by altering glucose homeostasis or promoting weight gain.

In turn, type 2 diabetes has been identified as a risk factor for the development of depression. Depressive symptoms and major depressive disorder are twice as prevalent in patients with type 2 diabetes as in the general population.[49]

Schizophrenia

Schizophrenia has been linked to the risk for type 2 diabetes. Dysfunctional signaling involving protein kinase B (Akt) is a possible mechanism for schizophrenia; moreover, acquired Akt defects are associated with impaired regulation of blood glucose and diabetes, which is overrepresented in first-episode, medication-naive patients with schizophrenia.[50]

Preeclampsia and Gestational Hypertension

A population-based, retrospective cohort study of 1,010,068 pregnant women examined the association between preeclampsia and gestational hypertension during pregnancy and the risk of developing diabetes post partum. Results showed the incidence rate of diabetes per 1000 person-years was 6.47 for

women with preeclampsia and 5.26 for those with gestational hypertension, compared with 2.81 in women with neither condition. Risk was further elevated in women with preeclampsia or gestational hypertension comorbid with gestational diabetes.[51]

Pathogenesis

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 non-diabetic, 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.[2]

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 progresses, 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.[2] This leads to a decrease in glucose transport into the liver, muscle cells, and fat cells. There is an increase in the breakdown of fat with hyperglycemia. The involvement of impaired alpha-cell function has recently been recognized in the pathophysiology of Type 2 DM.[21]

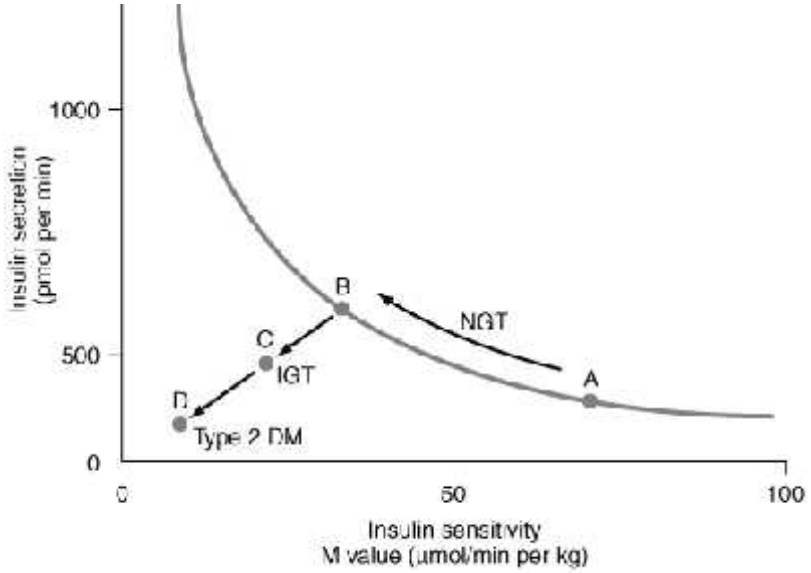


Figure 1. Metabolic changes during the development of type 2 diabetes mellitus[2]

As a result of this dysfunction, glucagon and hepatic glucose levels that rise during fasting are not suppressed with a meal. Given inadequate levels of insulin and increased insulin resistance, hyperglycemia results. The incretins are important gut mediators of insulin release, and in the case of GLP-1, of glucagon suppression. Although GIP activity is impaired in those with Type 2 DM, GLP-1 insulinotropic effects are preserved, and thus GLP-1 represents a potentially beneficial therapeutic option. However, like GIP; GLP-1 is rapidly inactivated by DPP-IV in vivo.[21]

Two therapeutic approaches to this problem have been developed: GLP-1 analogues with increased half-lives, and DPP-IV inhibitors, which prevent the breakdown of endogenous GLP-1 as well as GIP. Both classes of agents have shown promise, with potential not only to normalize fasting and postprandial glucose levels but also to improve beta-cell functioning and mass. Studies are ongoing on the role of mitochondrial dysfunction in the development of insulin resistance and etiology of Type 2 DM. Also very important is adipose tissue, as endocrine organ hypothesis (secretion of various adipocytokines, i.e., leptin, TNF-alpha, resistin, and adiponectin are implicated in insulin resistance and possibly beta-cell dysfunction).[21]

A majority of individuals suffering from Type 2 DM are obese, with central visceral adiposity. Therefore, the adipose tissue plays a crucial role in the pathogenesis of Type 2 DM. Although the predominant theory used to explain this link is the portal/visceral hypothesis giving a key role in elevated non-esterified fatty acid concentrations, two new emerging theories are the ectopic fat storage syndrome (deposition of triglycerides in muscle, liver and pancreatic cells). These two hypotheses constitute the framework for the study of the interplay between insulin resistance and beta-cell dysfunction in Type 2 DM as well as between our obesogenic environment and DM risk in the next decade.[21]

Screening[2]

Diabetes was originally identified by the presence of glucose in the urine. Almost 2,500 years ago it was noticed that ants were attracted to the urine of some individuals. In the 18th and 19th centuries the sweet taste of urine was used

for diagnosis before chemical methods became available to detect sugars in the urine. Tests to measure glucose in the blood were developed over 100 years ago, and hyperglycemia subsequently became the sole criterion recommended for the diagnosis of diabetes. Initial diagnostic criteria relied on the response to an oral glucose challenge, while later measurement of blood glucose in an individual who was fasting also became acceptable.

The most widely accepted glucose-based criteria for diagnosis are: fasting plasma glucose (FPG) ≥126 mg/dL or a 2-h plasma glucose ≥200 mg/dL during an oral glucose tolerance test (OGTT) on more than one occasion. In a patient with classic symptoms of diabetes, a single random plasma glucose ≥200 mg/dL is considered diagnostic. Before 2010 virtually all diabetes societies recommended blood glucose analysis as the exclusive method to diagnose diabetes. Notwithstanding these guidelines, over the last few years many physicians have been using HbA1C to screen for and diagnose diabetes. Although considered the "gold standard" for diagnosis, measurement of glucose in the blood is subjected to several limitations, many of which are not widely appreciated. Measurement of HbA1C for diagnosis is appealing but has some inherent limitations.[52]

These issues have become the focus of considerable attention with the recent publication of the Report of the International Expert Committee that recommended the use of HbA1C for diagnosis of diabetes,[53] a position that has been endorsed (at the time of writing) by the ADA,[54] the Endocrine Society, and in a more limited fashion by American Association of Clinical Endocrinologists/American College of Endocrinology.[55]

Current criteria for the diagnosis of diabetes:[56]

- HbA1C ≥6.5%. The test should be performed in a laboratory using a method that is National Glycohemoglobin Standardization Program (NGSP)-certified and standardized to the Diabetes Control and Complications Trial (DCCT) assay.
- Fasting plasma glucose (FPG) ≥126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h, or
- 2-h plasma glucose ≥200 mg/dl (11.1 mmol/l) during an oral glucose tolerance test (OGTT). The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.
- In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dl (11.1 mmol/l).
- In the absence of unequivocal hyperglycemia, result should be confirmed by repeat testing.

Oral Glucose Tolerance Test

The OGTT evaluates the efficiency of the body to metabolize glucose and for many years has been used as the "gold standard" for diagnosis of diabetes. An increase in postprandial glucose concentration usually occurs before fasting glucose increases. Therefore, postprandial glucose is a sensitive indicator of the risk for developing diabetes and an early marker of impaired glucose homeostasis. Published evidence suggests that an increased 2-h plasma glucose

during an OGTT is a better predictor of both all-cause mortality and cardiovascular mortality or morbidity than the FPG.[52]

The OGTT is accepted as a diagnostic modality by the ADA, WHO/International Diabetes Federation (IDF).[53] However, extensive patient preparation is necessary to perform an OGTT. Important conditions include, among others, ingestion of at least 150 g of dietary carbohydrate per day for 3 days prior to the test, a 10 to 16-h fast, and commencement of the test between 7:00 a.m. and 9:00 a.m.[52]

In addition, numerous conditions other than diabetes can influence the OGTT. Consistent with this, published evidence reveals a high degree of intra-individual variability in the OGTT, with a CV of 16.7%, which is considerably greater than the variability for FPG.[52] These factors result in poor reproducibility of the OGTT, which has been documented in multiple studies.[57] The lack of reproducibility, inconvenience, and cost of the OGTT led the ADA to recommend that FPG should be the preferred glucose-based diagnostic test.[53]

Advantages

- Sensitive indicator of risk of developing diabetes.
- Early marker of impaired glucose homeostasis

Disadvantages

- Lacks reproducibility
- Extensive patient preparation
- Time-consuming and inconvenient for patients

- Unpalatable
- Expensive
- Influenced by numerous medications
- Subject to the same limitations as FPG, namely, sample not stable, needs to be performed in the morning.

WHO Diabetes criteria - Interpretation of Oral Glucose Tolerance Test (1999)[58]

Glucose levels	**NORMAL**		**Impaired fasting glycaemia (IFG)**		**Impaired glucose tolerance (IGT)**		**Diabetes Mellitus (DM)**	
Venous Plasma	Fasting	2hrs	Fasting	2hrs	Fasting	2hrs	Fasting	2hrs
(mmol/L)	<6.1	<7.8	≥ 6.1 & <7.0	<7.8	<7.0	≥7.8	≥7.0	≥11.1
(mg/dL)	<110	<140	≥110 & <126	<140	<126	≥140	≥126	≥200

ADA criteria for diagnosis of diabetes mellitus[54]

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus of the American Diabetes Association stated that diabetes can be provisionally diagnosed with any one of the three criteria listed below. In the absence of unequivocal hyperglycemia with acute metabolic decompensation the diagnosis should be confirmed, on a subsequent day, by any one of the same three criteria.

- A fasting plasma glucose of >126 mg/dl (after no caloric intake for at least 8 hours) or,

- A random plasma glucose of >200 mg/dl (taken at any time of day without regard to time of last meal) with classic diabetes symptoms: increased urination, increased thirst and unexplained weight loss or,
- An oral glucose tolerance test (OGTT) (75 gram dose) of >200 mg/dl for the two hour sample. Oral glucose tolerance testing is not necessary if patient has a fasting plasma glucose level of >126 mg/dl.

The Committee states that the fasting plasma glucose is the preferred test and recommends moving toward its universal use for testing and diagnosis because of its ease of administration, convenience, acceptability to patients, and lower cost in comparison to the OGTT.

Summarized Interpretation of Oral Glucose Tolerance Test (OGTT)

- 2 hour postload glucose < 140 mg/dl = normal glucose tolerance
- 2 hour postload glucose between 140 mg/dl and 199 mg/dl = impaired glucose tolerance
- 2 hour postload glucose > 200 mg/dl = provisional diagnosis of diabetes (Must be confirmed on a subsequent day by any of the above criteria for diagnosis of Diabetes Mellitus.)

Standards of medical care in diabetes

Diabetes is a chronic illness that requires continuing medical care and patient education to prevent acute complications and to reduce the risk of long term complications.[24]

Initial Evaluation

Medical history

- Symptoms, results of laboratory tests, and special examination results related to the diagnosis of diabetes.
- Prior HbA1C records.
- Eating patterns, nutritional status, and weight history; growth and development in children and adolescents.
- Details of previous treatment programs, including nutrition and diabetes self-management education, attitudes, and health beliefs.
- Current treatment of diabetes, including medications, meal plan, and results of glucose monitoring and patients use of data.
- Exercise history.
- Frequency, severity, and cause of acute complications such as ketoacidosis and hypoglycemia.
- Prior or current infections, particularly skin, foot, dental, and genitourinary infections.
- Symptoms and treatment of chronic eye; kidney; nerve; genitourinary (including sexual), bladder, and gastrointestinal function (including symptoms of celiac disease in type 1 diabetic patients); heart; peripheral vascular, foot and cerebrovascular complications associated with diabetes.
- Other medications that may affect blood glucose levels.
- Risk factors for atherosclerosis: smoking, hypertension, obesity, dyslipidemia, and family history.

- History and treatment of other conditions, including endocrine and eating disorders.
- Assessment for mood disorder.
- Family history of diabetes and other endocrine disorders.
- Lifestyle, cultural, psychosocial, educational, and economic factors that might influence the management of diabetes.
- Tobacco, alcohol, and / or controlled substance use.
- Contraception and reproductive and sexual history.

Physical examination

- Height and weight measurements (and comparison to norms in children and adolescents).
- Sexual maturation staging (during pubertal period).
- Blood pressure measurement, including orthostatic measurements when indicated, and comparison to age-related norms.
- Fundoscopic examination.
- Oral examination.
- Thyroid palpation.
- Cardiac examination.
- Abdominal examination (e.g. for hepatomegaly).
- Evaluation of pulses by palpation and with auscultation.
- Hand/finger examination.
- Foot examination.
- Skin examination (for acanthosis nigricans and insulin-injection sites).
- Neurological examination.

- Signs of diseases that can cause secondary diabetes (e.g. hemochromatosis, pancreatic disease).

Laboratory evaluation

- HbA1C.
- Fasting lipid profile, including total cholesterol, HDL cholesterol, triglycerides, and LDL cholesterol.
- Test for microalbuminuria in type 1 diabetic patients who have had diabetes for at least 5 years and in all patients with type 2 diabetes; some advocate beginning screening of pubertal children before 5 years of diabetes.
- Serum creatinine in adults (in children if proteinuria is present).
- Thyroid stimulating hormone (TSH) in all type 1 diabetic patients; in type 2 if clinically indicated.
- Electrocardiogram in adults, if clinically indicated.
- Urinalysis for ketones, protein, sediment.

Referrals

- Eye exam, if indicated.
- Family planning for women of reproductive age.
- MNT, as indicated.
- Diabetes educator, if not provided by physician or practice staff.
- Behavioral specialist, as indicated.
- Foot specialist, as indicated.
- Other specialities and services as appropriate.

Recommendations for Adults with Diabetes

Summary of recommendations for adults with diabetes	
Glycemic control	
HbA1C	< 7.0%
Preprandial plasma glucose	90 – 130 mg/dl (5.0 – 7.2 mmol/l)
Postprandial plasma glucose	< 180 mg/dl (< 10.0 mmol/l)
Blood Pressure	< 130/80 mmHg
Lipids	
LDL	< 100 mg/dl (<2.6 mmol/l)
Triglycerides	< 150 mg/dl (<1.7 mmol/l)
HDL	> 40 mg/dl (> 1.1 mol/l)
Key concepts in setting glycemic goals : • Goals should be individualized. • Certain populations (children, pregnant women, and elderly) require special considerations. • Less intensive glycemic goals may be indicated in patients with severe or frequent hypoglycemia. • More stringent glycemic goals (i.e. a normal A1C < 6%) may further reduce complications at the cost of increased risk of hypoglycemia. • Postprandial glucose may be targeted if HbA1C goals are not met despite reaching preprandial glucose goals.	

Complications of Diabetes

Diabetes has both acute and long term complications.[2] They are:

Acute

- Diabetic ketoacidosis
- Hyperglycemic Hyperosmolar state
- Hypoglycemia

Long term:

- Retinopathy
- Neuropathy
- Nephropathy
- Ischemic heart disease
- Cerebrovascular disease
- Peripheral vascular disease
- Hypertensia.

Others

- ***Infections***
  - UTI
  - Tuberculosis
  - Candidiasis – oral / volvovaginal
  - Mucormycosis
  - Necrotising fasciitis
  - Periodontitis
- Dupuytrens contracture
- Pseudogout

Diabetic Retinopathy

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

Classification[59]

Non Proliferative Diabetic Retinopathy (NPDR)

1. *Mild NPDR*

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

2. *Moderate NPDR*

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

3. *Severe NPDR*

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

PDR

1. Early PDR

   One or more of the following:

   - NVE
   - NVD
   - Vitreous or preretinal hemorrhage
   - NVE< ½ disc area.

2. High risk PDR

   One or more of the following.

   - NVD > ¼- $^{1}/_{3}$ disc area

- NVD with vitreous or preretinal hemorrhage
- NVE > ½ disc area. Preretinal or vitreous hemorrhage.

3. Advanced PDR

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

- IRMA – Intraretinal microvascular abnormalities.
- NVE – Neovascularisation elsewhere.
- NVD – Neovascularisation disc.

Diabetic retinopathy progresses from mild non-proliferative abnormalities to moderate and severe non-proliferative diabetic retinopathy to proliferative diabetic retinopathy. Macular edema can develop at all stages of diabetic retinopathy. NPDR usually develops late in first decade or early 2nd decade of type – 2 diabetes mellitus. PDR usually develops within 5 years of NPDR. Pregnancy, poor glycemic control, hypertension and cataract surgery accelerate these changes. UKPDS study revealed that for every percentage reduction of HbA1C (eg. From 8 to 7%), there was a 35% reduction in risk of retinopathy,[19] and tight BP control (to < 150/85 mmHg) results in 34% reduction in progression of retinopathy.[60]

Diabetic Nephropathy

Diabetes has become the most common single cause of endstage renal disease (ESRD) world wide. About 20-30% of patients with type 1 or type 2 diabetes mellitus develop evidence of nephropathy, but in type 2 diabetes a

considerably smaller fraction of these progress to ESRD. However, because of much higher prevalence of type 2 diabetes mellitus, these patients constitute over half of patients with nephropathy needing dialysis.[24]

The diabetic nephropathy progresses from appearance of low but abnormal levels of (≥ 30mg to 299 mg/day or 20µg/min) albumin in urine (stage of microalbuminuria) to stage of macroalbuminuria / clinical albuminuria (≥300mg/day or ≥200µg/min) to ESRD. Progress from microalbuminuria to macroalbuminuria usually takes 10-15 yeas. ESRD develops in 50% of type 1 diabetic individuals with clinical nephropathy within 10 years and in 75% by 20 years. But in type 2 diabetes mellitus, even after 20 years of overt nephropathy only 20% progress to ESRD.

Screening for Microalbuminuria:

A test for the presence of urinary microalbumin should be performed at diagnosis in patients with type 2 diabetes mellitus and after 5 years of disease duration in those with type 1 diabetes mellitus, then repeated annually. Screening for microalbuminuria can be performed by three methods.

1. Measurement of the albumin to creatinine ratio in a random spot urine collection.
2. 24 hr Urine collection and measurement of albumin excretion.
3. Timed (e.g. 4 hr or overnight) collection.

24 hr collection of urine is most reliable.

Definitions of abnormalities in albumin excretion[24]

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

In addition to being the earliest manifestation of nephropathy, albuminuria is a marker of greatly increased cardiovascular morbidity and mortality in patients with either form of diabetes.[60]

Diabetic Neuropathy

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

Classification

Symmetric

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

Asymmetric

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

Also classified as follows (Watkin's and Edmond's classification)[61]

1. Progressive Neuropathies
    - Chronic sensory motor neuropathy
    - Autonomic neuropathy
2. Reversible Neuropathies
    - Mononeuropathies
        - Proximal motor neuropathy (Amyotrophy)
        - Cranial nerve palsies (III,IV,VI)
        - Truncal radiculopathies
    - Acute painful neuropathies
3. Pressure Palsies
    - Carpal tunnel syndrome.

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

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

Cardiovascular Disease in Diabetes

Cardiovascular disease incidence is increased in individuals with type 1 or type 2 diabetes mellitus. The Framingham Heart Study revealed a marked increase in peripheral arterial disease, coronary artery disease, myocardial infarction, congestive heart failure and sudden death (risk increases from one to five fold) in diabetes mellitus.[62] The American Heart Association recently designated type 2 diabetes mellitus as a coronary risk equivalent i.e. they have a similar 10 year risk of MI, as those who have had a prior MI.[24] Because of extremely high prevalence of underlying cardiovascular disease in patients with type 2 diabetes mellitus, evidence of atherosclerotic vascular disease should be sought in a diabetic who has symptoms suggestive of cardiac ischemia, peripheral or carotid artery disease, a resting ECG indicative of prior MI, plans to initiate an exercise program, proteinuria or two other cardiac risk factors. (ADA recommendations).[24]

In addition to coronary artery disease, cerebrovascular disease is increased in individuals with diabetes mellitus (three fold increase in stroke). Proof that improved glycemic control reduces cardiovascular complications in diabetes mellitus is lacking.[63]

Overall, diabetes is a metabolic disease that can affect nearly every organ system in the body. Diabetes continues to be a public health concern. Although important knowledge has been acquired on the etiology of diabetes its precise etiopathogenesis is still under discussion. Inflammatory factors, reactive oxygen species and autoimmune reactions have all strongly emerged as the major pathogenic effectors for diabetes. Recently, Vitamin D has sparked widespread interest in the pathogenesis and prevention of diabetes. As the major regulator for calcium homeostasis, Vitamin D directly and or indirectly improves insulin exocytosis via activating calcium-dependent endopeptidases. Vitamin D also improves glucose tolerance.[64] Vitamin D could also prevent type 2 diabetes through its role as an efficient antioxidant.

Additionally, the steroid hormone form of Vitamin D promotes suppressor cell activity and inhibits the generation of cytotoxic (Tc), macrophages, delayed hypersensitivity type and natural killer (NK) cells.[65] Vitamin D also mediates several non-calcemic functions. It is a regulator of cellular proliferation, differentiation and replication, and mediator of autoimmune reactions, in a variety of organs and biological systems. The discovery of receptors for 1α, 25-dihydroxyvitamin D3 (1,25(OH)2D3), the activated form of Vitamin D, in tissues with no direct role in calcium and bone metabolism (e.g.,

pancreatic beta-cells and cells of the immune system) has broadened our view of the physiological role of Vitamin D.

Vitamin D

Vitamin D (VD) was first identified and characterized in 1923 by Goldblatt and Soames.[67] It is an essential vitamin naturally produced by the body on exposure to sunlight.

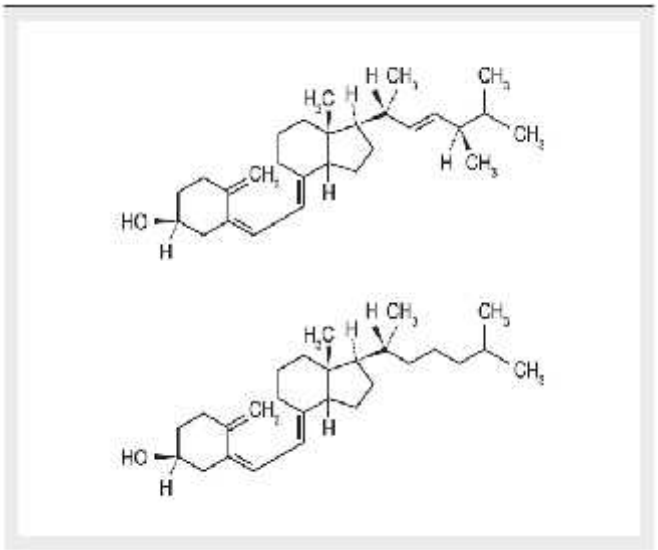


Figure 2. Molecular structures of upper vitamin D2 (ergocalciferol) and lower vitamin D3 (Cholecalciferol)[20]

The term Vitamin D encompasses secosterols, ergocalciferol (VD2) and cholecalciferol (VD3). Vitamin D2 is produced commercially by irradiation of plant sterols (ergosterol), whereas Vitamin D3 is primarily manufactured in the skin from 7-dehydro cholesterol via photochemical synthesis using UV radiation from sunlight and can also be found in food of animal origin. The best food sources of Vitamin D are cod liver oil, fatty fish, and egg yolks.

Metabolism of Vitamin D

Natural Vitamin D by itself has no hormonal activity. To become biologically active, Vitamin D needs two successive hydroxylations one in the liver (at carbon 25) and another in the kidney (at a position of carbon 1). In the liver Vitamin D is hydroxylated at carbon 25 to 25-hydroxy Vitamin D (25(OH)D). Circulating 25(OH)D concentrations are considered an indicator of Vitamin D status. In the kidneys, 25-hydroxy Vitamin D (25(OH)D) is converted to an activated (1,25-dihydroxy vitamin D; 1,25(OH)2D). This is the biologically active form of Vitamin D.[20]

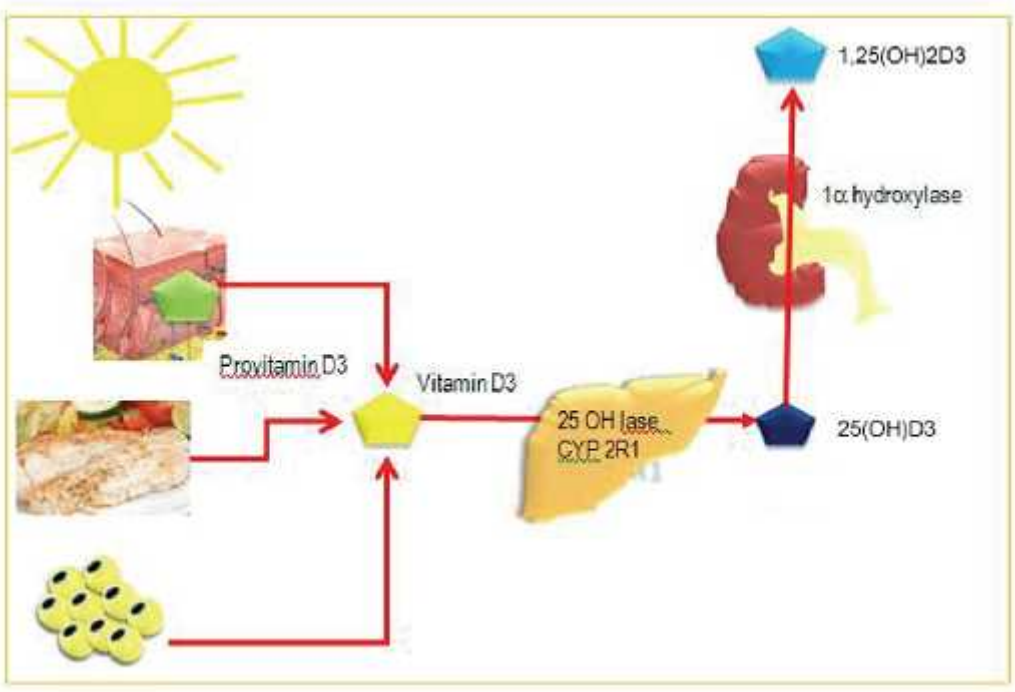


Figure 3. Synthesis and metabolism of Vitamin D. Vitamin D can be obtained from food (vitamin D2 and D3) or by photobiogenesis in the skin (vitamin D3). In the blood, all Vitamin D metabolites are bound to Vitamin D-binding protein (DBP). Vitamin D3 is converted by two successive hydroxylations in the liver (25-hydroxylases) and kidney (1α-hydroxylase) into its active hormonal form, 1,25(OH)2D3.[20]

The production of 1,25(OH)2D3 in the kidney is regulated by several factors, particularly by levels of parathyroid hormone, although kidney 1α-hydroxylase is also subject to direct negative feedback inhibition by 1,25(OH)2D3. The proximal renal tubule is the principal site of 1α-hydroxylation, although high levels of 1α-hydroxylase mRNA have also been found in human keratinocytes, dendritic cells and macrophages.

Another hydroxylation enzyme, 24-hydroxylase, initiates the catabolic cascade of 25-hydroxyvitamin D3 and 1,25(OH)2D3. In the circulation, all metabolites of vitamin D are bound to a carrier protein known as Vitamin D-binding protein (DBP).[66]

Molecular action of 1,25-dihydroxyvitamin D3

The Vitamin D hormone exerts its effects mainly by activating the nuclear Vitamin D receptor (VDR), a member of the nuclear receptor super-family of ligand activated transcription factors. In humans the gene encoding the VDR is located on chromosome 12cen-q12. The binding of 1,25(OH)2D3 to the VDR leads to the transcription of genes regulated by 1,25(OH)2D3. The mechanism of this transcriptional regulation is very complex and is only just beginning to be unravelled.

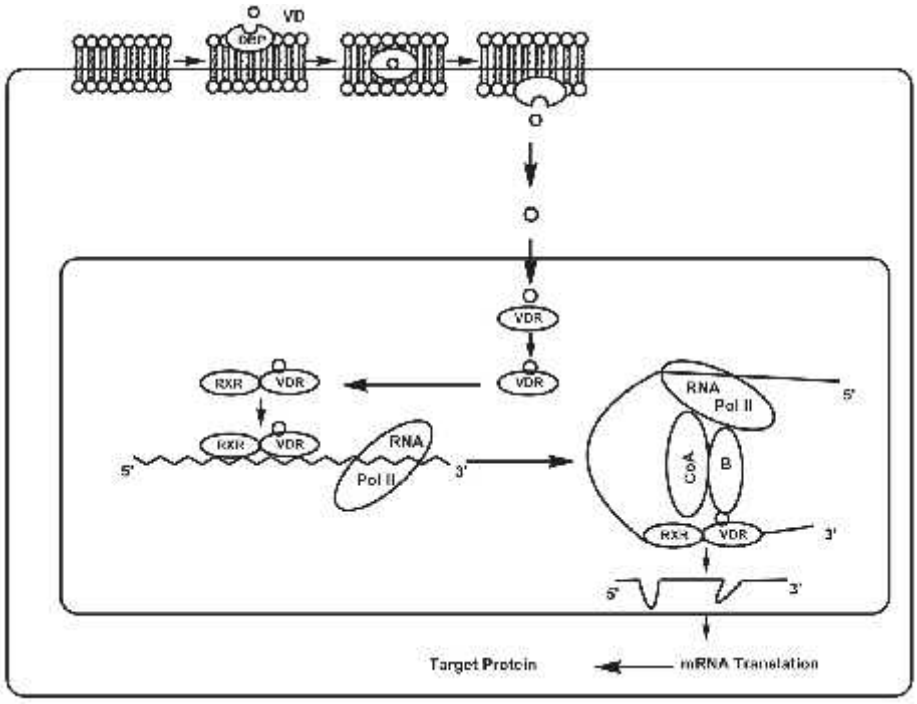


Figure 4. Genomic actions of 1,25-dihydroxyvitamin D3[20]

Molecules of 1,25(OH)2D3 penetrate the plasma membrane with the help of DBP and exert their genomic effects by activating the VDR. Ligand binding to the VDR induces a conformational change in the receptor and subsequent heterodimerization with RXR. The RXR-VDR complex binds to the VDRE, which is located within the 5' flanking region of target genes. Thereafter, co-repressor (CoR) proteins are released from the surface of the VDR, allowing interaction with co-activator (CoA) proteins. These molecules modulate chromatin structure and allow the interaction of the receptor with the RNA polymerase II transcriptional complex (POL II), thus activating transcription of the target gene.

The cognate Vitamin D response element (VDRE) to which the VDR binds consists of a hexanucleotide direct repeat spaced by three nucleotides

(DR3-type VDRE). The VDR usually binds as a heterodimer with the retinoic X receptor (RXR), and the classical effects of 1,25(OH)2D3 are the result of interactions with this nuclear receptor.

With respect to its relevance in diabetes, the classical VDRE and other response sites are found within genes encoding proteins with important functions in beta-cells and within genes encoding proteins with key roles throughout the immune system, such as cytokines and transcription factors.[68]

Vitamin D deficiency

Serum 25(OH)D is the best indicator of Vitamin D body store levels. The desirable concentration of Vitamin D in normal healthy adult should be greater than 100 nmol/l. Vitamin D deficiency is characterized by circulating levels of 25(OH)D less than 50 nmol/l. Concentrations ranging between 52-72 nmol/l are often considered insufficient.[69]

Vitamin D deficiency is a common problem and the clinical consequences are protean. Low Vitamin D status can be caused by number of factors, including insufficient cutaneous synthesis (due to limited sunlight exposure or aging), inadequate intake and absorption of Vitamin D, obesity or darker skin. Low blood levels of its main metabolite, 25(OH) D, have been linked to poor health outcomes such as fractures, poor physical function, sarcopenia, diabetes, osteoporosis, cancer, cardiovascular, neurodegenerative, autoimmune and infectious diseases.[70]

Vitamin D and Type 2 Diabetes

Type 2 diabetes is characterized by insulin resistance and altered insulin secretion. The role of Vitamin D in type 2 diabetes is suggested by a seasonal variation in glycemic control reported in patients with type 2 diabetes, being worse in the winter,[66] which may be due to prevalent hypovitaminosis D as a result of reduced sunlight in winter. Several studies have demonstrated a link between Vitamin D and the incidence of type 2 diabetes. In a recent study, de Boer et al,[71] examined the effect of calcium plus Vitamin D supplementation on the incidence of drug-treated diabetes in postmenopausal women and concluded that, calcium plus Vitamin D3 supplementation did not reduce the risk of developing diabetes over seven years of follow-up in this randomized, placebo controlled trial. However, they suggested that, higher doses of Vitamin D might be required to affect diabetes risk. In support of this argument, in Nurses Health Study – a large prospective, observational cohort, women with the highest calcium and Vitamin D intake (> 1200 mg and > 800 IU daily, respectively) had a 33% lower risk of incident type 2 diabetes mellitus than women with the lowest calcium and Vitamin D intake (< 600 mg and < 400 IU daily, respectively).[72]

Another large cohort study from Finland indicated inverse association between serum 25(OH)D3 and risk of type 2 diabetes. These results were consistent with those from the Nurses' Health Study by Pittas et al,[65] where an inverse association was observed for the intake of Vitamin D supplements.

However, these studies were not able to differentiate whether the results were due to the effect of Vitamin D deficiency on beta-cell function or on insulin

resistance. Several reports have ascribed an active role to Vitamin D in the functional regulation of the endocrine pancreas, particularly the beta-cells. Not only are receptors for 1,25(OH)2D3 found in beta-cells, but the effector part of the Vitamin D pathway is also present in the form of Vitamin D-dependent calcium binding protein, also known as calbindin-D28k. The expression of calbindin-D28K has been shown to protect beta-cells from cytokine-mediated cell death,[66] thereby reducing the risk of type 2 diabetes.

Mechanisms of action of Vitamin D on Type 2 diabetes

Vitamin D and the beta-cell

There are several lines of evidence supporting a role for Vitamin D in pancreatic beta-cell function. Sheena et al[73] examined the cross-sectional association between vitamin D and beta-cell dysfunction in subjects at risk for type 2 diabetes and showed a positive association between Vitamin D and beta-cell function. A high prevalence of hypovitaminosis D was noted among women with type 2 diabetes. Hyper responsive insulin secretion after a glucose challenge has been found in older men with hypovitaminosis D.[74]

Vitamin D may act in two possible pathways; Vitamin D may act directly to induce beta-cell insulin secretion by increasing the intracellular calcium concentration via non-selective voltage-dependent calcium channels or it may mediate activation of beta-cell calcium-dependent endopeptidases to produce the cleavage that facilitates the conversion of proinsulin to insulin. In peripheral insulin-target tissues, Vitamin D might directly enhance insulin action through

stimulation of the expression of insulin receptors and regulation of insulin-mediated intracellular processes via regulation of the calcium pool.[66]

Insulin resistance

Insulin resistance is a recognized precursor for the development of type 2 diabetes. Vitamin D may have a beneficial effect on insulin action either directly, by stimulating the expression of insulin receptors thereby enhancing insulin responsiveness for glucose transport, or indirectly via its role in regulating extracellular calcium ensuring normal calcium influx through cell membranes and adequate intracellular cytosolic calcium [Ca2+]i pool. Calcium is essential for insulin-mediated intracellular processes in insulin-responsive tissues such as skeletal muscle and adipose tissue with a very narrow range of [Ca2+]i needed for optimal insulin-mediated functions. Changes in [Ca2+]i in primary insulin target tissues may contribute to peripheral insulin resistance via impaired insulin signal transduction leading to decreased GLUT-4 activity.[20]

Associations between low Vitamin D level and decreased insulin sensitivity have been reported in cross-sectional studies. A recent study by Enju Liu et al[69] examined the association between Vitamin D status and insulin resistance in non-diabetic individuals and showed that higher Vitamin D status was inversely associated with fasting markers of insulin resistance.

A positive correlation of 25(OH)D concentration with insulin sensitivity and a negative effect of hypovitaminosis D have been reported by Ken C Chiu et al.[75]

Some observational[71,75] studies have shown an inverse association between Vitamin D-calcium status and insulin resistance. Results from randomized trials on the effect of Vitamin D and/or calcium supplementation on insulin resistance show either improvement[75-77] or no effect[78] of insulin action with supplementation.

Inflammation

Type 2 Diabetes is associated with systemic inflammation. Systemic inflammation has been linked primarily to insulin resistance but elevated cytokines may also play a role in beta-cell dysfunction by triggering beta-cell apoptosis. Vitamin D may improve insulin sensitivity and promote beta-cell survival by directly modulating the generation and effects of cytokines. Vitamin D interacts with Vitamin D response elements in the promoter region of cytokine genes to interfere with nuclear transcription factors implicated in cytokine generation and action. Vitamin D can down regulate the activation of NF-kB, which is an important regulator of genes encoding pro-inflammatory cytokines implicated in insulin resistance. Vitamin D interferes with cytokine generation by upregulating expression of calbindin, a cytosolic calcium-binding protein found in many tissues including pancreatic beta-cells. Calbindin has been shown to protect against cytokine-induced apoptosis that may occur after a rise in cytosolic free calcium [Ca2+]I.[20] There is very limited and conflicting data from human studies that has directly examined the relationship between Vitamin D or calcium status and systemic inflammation in relation to type 2 Diabetes.[65]

Vitamin D and Glucose Transport

Insulin stimulates glucose metabolism in its target tissues via recruitment of transporters from a large intracellular pool to the plasma membrane. The functional activity of these transporters has been shown to be impaired in diabetes. As Vitamin D modulates insulin secretion, it is reasonable that Vitamin D deficiency may in part contribute to the altered expression of these transporters. Although the role of Vitamin D in diabetes is well recognized, its relation to glucose transport is not well studied.[20]

Consuelo et al[79] observed the effect of Vitamin D on glucose transport in adipocytes in non-diabetic and streptozotocin induced diabetic rats. Treatment with 1,25(OH)2D3 to non-diabetic rats did not alter basal and insulin stimulated glucose transport in adipocytes from these animals. The treatment with Vitamin D to streptozotocin induced diabetic rats, improved the decreased basal glucose transport by 107% and the insulin stimulated glucose transport in adipocytes by 71% from these diabetic animals. The Possible mechanism is likely to be the indirect effect of Vitamin D. Vitamin D contributes to normalization of extracellular calcium ensuring normal intracellular calcium pool as elevated intracellular calcium impairs insulin receptor phosphorylation leading to impaired insulin signal transduction and decreased GLUT-4 activity.

Peeyush et al[80] examined the effect of Vitamin D supplementation on preventing the altered expression of GLUT-3 in STZ-induced diabetic rats leading to imbalanced glucose transport in the neurons of cerebellum. They observed that treatment with Vitamin D and insulin, stabilized the glucose

transport mechanism mediated through GLUT-3 in the cerebellum. They concluded that supplementation with Vitamin D to STZ-induced diabetic rats has beneficial effects in reducing the alterations in GLUT-3 and imbalanced glucose utilization in cerebellum. However large, well-controlled, randomized studies are required to define the relationship between Vitamin D and glucose transport.

Vitamin D and Complications of Diabetes

Diabetes is associated with complications such as cardiovascular disease, renal impairment, and peripheral neuropathies. Studies in the past have explored the relationship of Vitamin D concentrations to these complications. The earliest evidence for the relationship of Vitamin D concentration to cardiovascular disease began with diabetic patients who had end stage renal disease. It was found that in patients having dialysis the cardiovascular mortality was 10 to 20 times higher than the general population. The severe hypertension in diabetic patients was attributed to the reduced synthesis of calcitriol by the kidneys. When patients were given 1 alpha-vitamin D and the Vitamin D analogue paricalcitol, it was noted that the mortality rate for cardiovascular disease was significantly reduced.[81]

A study on 825 US hemodialysis patients to determine the relationship between Vitamin D levels and mortality reported that 78% of the patients were Vitamin D deficient and that this was associated with an increased early mortality.[82]

Zhang et al[83] reported that receptor-mediated Vitamin D actions may be protective of kidneys in rats with diabetic nephropathy. This suggests that Vitamin D may be useful and preventative for the kidneys.

In a study to determine the relationship between 25(OH) D and kidney functions on NHANES III participants, the level of 25(OH)D was significantly lower in persons with severely decreased glomerular filtration rate when compared with those with normal kidney function.[84]

Diabetes has been associated with several neurological disorders including reduced locomotor activity which in turn is associated with low concentration of Vitamin D.[85]

A study by Peeyush KT[86] reported the altered expression of cholinergic and dopaminergic receptors in the central nervous system of STZ-induced diabetic rats. They further suggested that the altered expression of these receptors was brought back to control by the treatment with Vitamin D.

A small, clinical study of 51 patients with type 2 diabetes (37 female) evaluated neuropathic complaints such as pain, burning, tingling, numbness, and throbbing sensations. Patients who were Vitamin D deficient at base line were given cholecalciferol (D3) and re-evaluated at 3-month follow-up. Serum concentrations increased 67.4% from 18 to 30 ng/mL, and were associated with significantly lower pain scores.[87] These findings provide a confirmatory evidence for neuroprotective role of Vitamin D and represent a novel possibility for the better management of diabetic mediated complications.

Overall, the published literature supports a possible role of Vitamin D in the pathogenesis and prevention of diabetes. Vitamin D deficiency appears to be detrimental to beta-cell function, and leads to glucose intolerance in animal models and humans, and consequently type 2 diabetes. Vitamin D deficiency in early life predisposes NOD mice and humans to the later development of autoimmune diabetes. Several Vitamin D related genes have shown association with different pathogenetic traits of the disease. Vitamin D and its related metabolic and immune pathways may be involved in the pathogenesis of diabetes at environmental and genetic levels.[20]

Recent Literature on Role of Vitamin D in Diabetes Mellitus

The effect of Vitamin D supplementation was studied by Avanell et al.[88] In sum, 7 out of 8 studies found a significant association between diabetes and Vitamin D, and the study from Avanell et al[88] didn't rule out a protective role of Vitamin D.

In a cross-sectional study from Renzaho et al[89] the demographics were described of 49 African migrants living in Melbourne. In 88% of the participants Vitamin D insufficiency occurred, and the participants were at high risk for developing type 2 diabetes or cardiovascular disease. The risk was measured by measuring the following factors: being overweight, hypertension, insulin resistance and cholesterol. Considering the voluntary nature of the study, and the small number of participants, the results cannot be easily generalized to a bigger community. The authors suggest that a study with Vitamin D intervention can

give more evidence for the existing relationship between Vitamin D and type 2 diabetes.

In a cross-sectional population based study from Shankar et al[90] 12,719 US adults who filled out the National Health and Nutrition Examination Survey were used. Adjustments were made for age, sex, season, race, smoking, geographic region, alcohol intake, BMI, physical activity, Vitamin D intake, hypertension and cholesterol. Compared with the highest quartile, the odds ratio of pre-diabetes for the lowest quartile was 1.47 (1.16–1.85) p = 0.001. The conclusion from this study is; low levels of Vitamin D are associated with pre-diabetes in US adults.

In a longitudinal cohort study in 4,097 Finland survey participants examined by Mattila et al[91] the relation between serum 25(OH)D levels and subsequent risk of type 2 diabetes was observed. Adjustments were made for age, sex and month when the blood samples were collected. After a follow-up of 17 years the relative risk of diabetes for the highest quartile compared to the lowest quartile of serum 25(OH)D was 0.60 (0.36-0.98) p=0.01, showing that higher levels of 25(OH)D were associated with a significant decreased diabetes risk. Thus high Vitamin D levels are good. When further adjustments were made for BMI, smoking, education and exercise the relative risk was no longer significant: 0.70 (0.42-1.16) p=0.07. These findings showed a significant positive association between high levels of Vitamin D and decreased risk of developing type 2 diabetes.

Liu et al[92] used a subsample of the Framingham Offspring Study to examine the relation between Vitamin D status and incidence of type 2 diabetes. Adjustments were made for age, sex, waist circumference, parental history of type 2 diabetes, hypertension, cholesterol, impaired fasting glucose and diet. In this prospective cohort study, the plasma 25(OH) D levels from 3066 participants were collected. In total 133 participants developed type 2 diabetes after 7 years. Compared to the lowest quartile, those in the highest quartile of predicted 25(OH) D levels had a 40% lower incidence for developing type 2 diabetes HR: 0.60 (0.37- 0.97) (p = 0.03). They suggested that maintaining the Vitamin D status can lower the risk of developing type 2 diabetes.

The correlation between sun exposure and type 2 diabetes was analyzed by Lindqvist et al[93] in a prospective cohort study. During an 11 year follow-up 24,098 Swedish women answered an inquiry. Women with active sun exposure were at 30% less risk for developing type 2 diabetes, compared to the women with non-active sun exposure. Also the women with more sun exposure had a lower risk (ranging from 40-60%) of developing type 2 diabetes. A possible explanation is that BMI is a high risk for type 2 diabetes, and Vitamin D influences this. Overall, this study supports that sunlight is involved in glucose metabolism, and BMI appears to mediate this association.

In a prospective population based study from Grimnes et al[94] 6119 Norwegian citizens were included. During an 11 year follow-up period, the risk of developing type 2 diabetes was measured in relation to Vitamin D levels. Adjustments were made for age, sex, BMI, physical activity and smoking behavior. Vitamin D levels were measured every month, and diabetes was

defined using a hospital journal-based end-point registry. The risk for the development of diabetes in the highest quartile was used as reference, the hazard ratios for the third, second and first quartiles were: non smokers: 1.00 (0.62–1.61), 1.50 (0.97–2.31) and 1.89 (1.25–2.88), smokers: 1.79 (0.77–4.19), 2.33 (1.02–5.35) and 2.68 (1.18–6.08). In this analysis the ratios were adjusted for age and sex. Adjustments for BMI rendered the findings nonsignificant. The conclusion of this study is that the risk of developing type 2 diabetes increases when Vitamin D levels are low, but this may be partially mediated by, once again, BMI.

Pittas et al[95] examined 83,779 female nurses in a prospective study. The purpose of this study was to determine the relation between Vitamin D and calcium intake and the risk of developing type 2 diabetes. During a 20 year follow-up 4,843 cases of type 2 diabetes were documented. Adjustments were made for age, sex, smoking, BMI, physical activity, family history of diabetes, hypertension and alcohol consumption. The RR for type 2 diabetes was 0.87 (0.75–1.00) $p = 0.04$, comparing the highest with the lowest category of Vitamin D from supplements assessment. When adjusted for the confounding factors, no relationship was found. However, the relative risk for type 2 diabetes was: 0.67 (0.49–0.90) when comparing the daily intake of Vitamin D >800 IU and calcium 1,200 mg versus <400 IU Vitamin D and 600 mg calcium. Thus Vitamin D and calcium may play a role in the development of type 2 diabetes.

Eight articles were discussed regarding the relation between Vitamin D and diabetes. Two cross-sectional studies from Renzaho et al[89] and Shankar et al[90] found that low Vitamin D levels have been associated with risk of having

diabetes. The prospective studies from Pittas et al,[95] Liu et al[92] and Lindqvist et al[93] found a higher risk of developing diabetes when Vitamin D levels are low. Mattila et al[91] found a significant inverse association between Vitamin D and diabetes in a longitudinal design. These findings from Mattila support the statement that Vitamin D insufficiency leads to and precedes diabetes. Grimnes et al[94] found a significant higher risk of developing diabetes when Vitamin D was low, but not if controlled for BMI. The intervention study from Avanell et al[88] found no significant effect from Vitamin D supplementation on the risk of developing type 2 diabetes mellitus. The authors noted that the Vitamin D supplements didn't contain high levels of Vitamin D, which may have influenced the results.

Overall, studies on Vitamin D supplementation on prevention of diabetes are inconclusive. However robust clinical data is required to support a role for Vitamin D supplementation in the prevention of diabetes.

Chapter 4

Methodology

METHODOLOGY

The present study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on patients with type 2 diabetes mellitus during the study period from January 2012 to December 2012

Study design

The study design was one year cross sectional study.

Study period and duration

The present one year study was conducted from January 2012 to December 2012.

Place

The present study was conducted in Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum a tertiary care hospital attached to Jawaharlal Nehru Medical College, Belgaum.

Source of Data

Patients with type 2 diabetes mellitus admitted in the wards of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum were studied.

Sample size

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

Sampling procedure

Considering the recent literature on prevalence of vitamin D deficiency in patients with type 2 diabetes mellitus which has shown more than half of subjects with vitamin D deficiency, 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 x 50 x 50 / 100

n = 100

Hence a sample size of 100 patients with type 2 diabetes mellitus was considered.

Selection criteria

Inclusion criteria

- Patients with type 2 diabetes mellitus.
- Age more than 18 years.

Exclusion criteria

- Type 1 diabetic patients.
- Liver failure.
- Kidney failure.
- Patient on treatment with Vitamin D

Ethical clearance

The ethical clearance was obtained from Institutional Ethics Committee, Jawaharlal Nehru Medical College, Belgaum before the commencement of the study.

Informed Consent

The patients fulfilling selection criteria were explained about the nature of the study. Those willing to participate were enrolled in the study 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, medication, personal history and history pertaining to the other comorbid conditions was obtained. Further these patients were subjected to a thorough physical examination such as anthropometry (including height and weight), vitals (pulse rate, blood pressure and respiratory rate) and systemic examination. These findings were recorded on a predesigned and pretested proforma (Annexure II).

Investigations

The selected patients underwent the following investigations.

- Complete blood count.
- Mini renal profile.
- Liver function test.
- Urine – Routine and microscopy.
- Blood sugar levels – Fasting and post prandial
- Glycated haemoglobin (HbA1c)
- Fundoscopy
- Fasting lipid profile
- Fasting plasma insulin
- Fasting Serum 25-hydroxy Vitamin D

Fasting Serum 25-hydroxy Vitamin D

The estimation of Vitamin D was done using Siemens, ADVIA Centraur VitD assay, a one pass 18 minute antibody competitive immunoassay that used an anti fluorescein monoclonal mouse antibody covalently bound to paramagnetic particles and anti 25 (OH) Vitamin D monoclonal mouse antibody labeled with acridinium ester and a Vitamin D analog labeled with fluorescein.[96]

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.[97]

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

Lipid profile

Total cholesterol, triglycerides, HDL, LDL levels were noted and the findings were recorded. Normal values of lipid parameters were interpreted as;

- Low density lipoprotein < 100 mg/dL.
- High density lipoprotein < 40 mg/dL
- Total Cholesterol < 200 mg/dL.
- Triglycerides < 150 mg/dL.

Fasting Serum 25-hydroxy Vitamin D

The results were interpreted as below.

- < 50 nmol/L – Deficiency
- ≥ 50 to 75 nmol/L – Insufficiency
- > 75 nmol/L – Normal

Statistical analysis

The data obtained was coded and entered into Microsoft Excel Worksheet (Annexure III). 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 comparison was done by two sample 't' test with unequal variance. A probability value ('p' value) of less than or equal to 0.05 was considered as statistically significant.

Chapter 5

Results

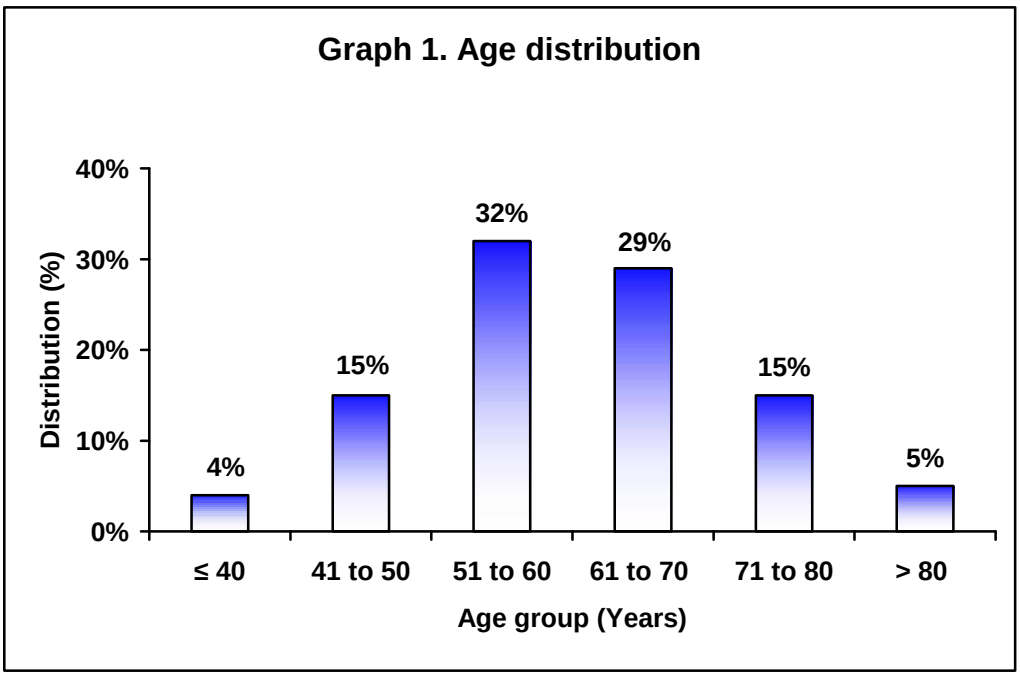
RESULTS

The present one year cross sectional study titled Estimation of levels of Vitamin-D in Type 2 Diabetes Mellitus was carried out in the Department of Medicine, 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 were studied. The findings/observations and final results are tabulated as below.

Table 1. Age distribution

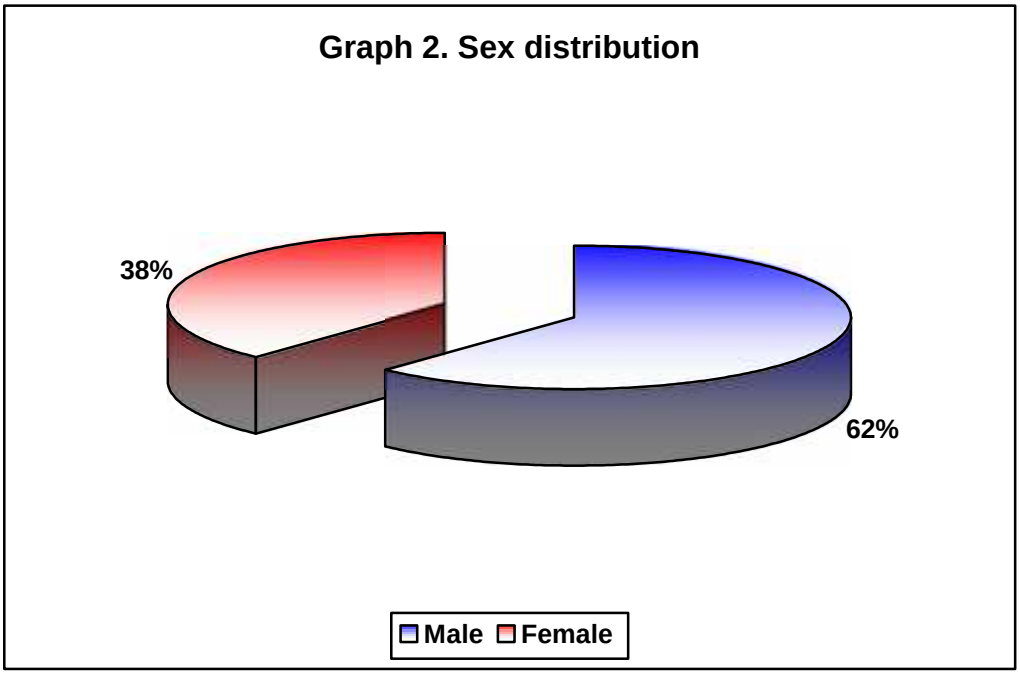
Age group (Years)	Distribution (n=100)	
	Number	**Percentage**
≤ 40	4	4.00
41 to 50	15	15.00
51 to 60	32	32.00
61 to 70	29	29.00
71 to 80	15	15.00
> 80	5	5.00
Total	**100**	**100.00**



Patients age ranged from 39 to 85 years, maximum number of cases were in the age group of 51 to 60 that is 32 patients (32%), between 61 to 70 years 29 patients (29%) and between 41 to 50 years and 71 to 80 years 15 patients (15%) in each group. The mean age of study population was 60.6 ± 11.28 years.

Table 2. Sex distribution

Sex	Distribution (n=100)	
	Number	**Percentage**
Male	62	62.00
Female	38	38.00
Total	**100**	**100.00**



Out of 100 patients, 62 (62%) were males and 38 patients (38%) were females, accounting a ratio of male to female 1.63:1.

Inference: Male preponderance was observed.

Table 3. Duration of diabetes

Duration (years)	Distribution (n=100)	
	Number	**Percentage**
Upto 5	32	32.00
6 to 10	33	33.00
11 to 15	16	16.00
> 15	19	19.00
Total	**100**	**100.00**

In the present study, we observed in 33% of patients the duration of diabetes was 6 to 10 years. In 32% of patients the duration of diabetes was either less than 5 years or upto 5 years. In 19% of patients the duration of diabetes was >15 years. The mean duration of diabetes was 9.70 ± 6.90 years.

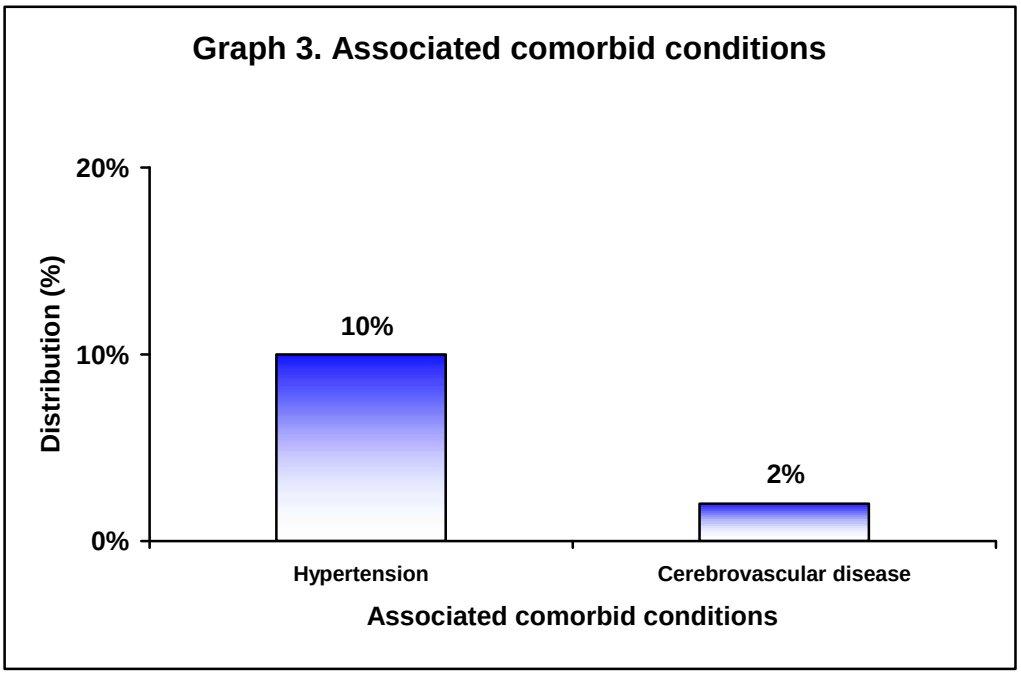
Table 4. Mode of treatment

Treatment	Distribution (n=100)	
	Number	**Percentage**
Oral hypoglycaemic agents	64	64.00
Insulin	36	36.00
Total	**100**	**100.00**

We observed 64 patients (64%) were on oral hypoglycaemic agents and 36 patients (36%) were on insulin preparations.

Table 5. Associated comorbid conditions

Comorbid conditions	Distribution (n=100)	
	Number	Percentage
Hypertension	10	10.00
Cerebrovascular disease	2	2.00



In the present study, 10 patients (10%) had hypertension. 2 patients (2%) had cerebrovascular accidents.

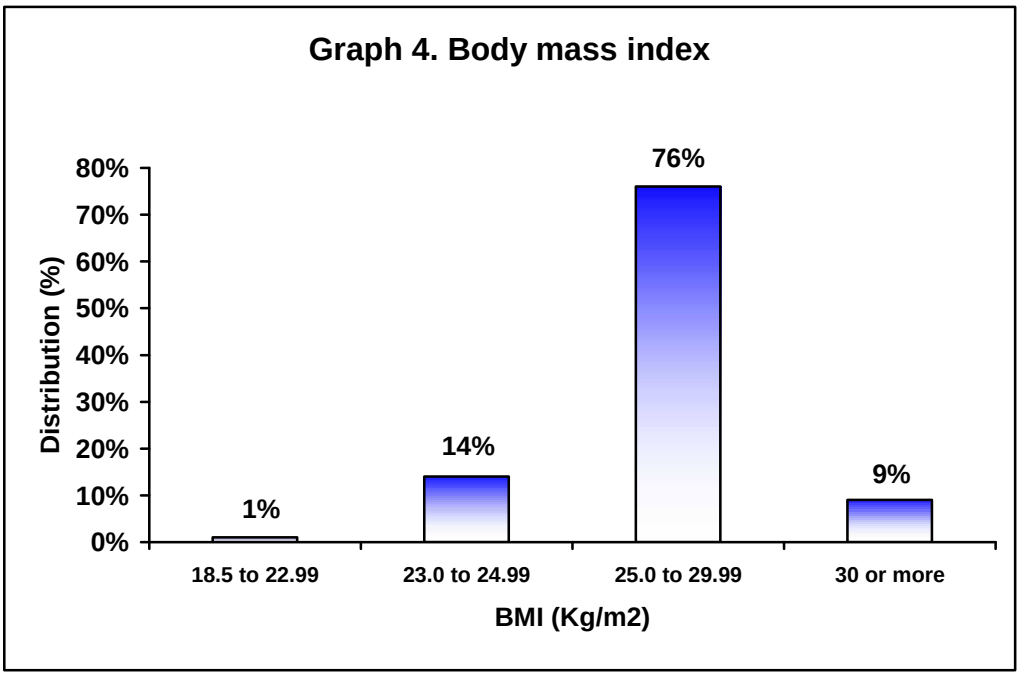
Table 6. Habits

Habits	Distribution (n=100)	
	Number	**Percentage**
Alcohol	14	14.00
Tobacco consumption	9	9.00
Smoking	8	8.00

In 14 patients (14%) history of alcohol consumption, 9 patients (9%) history of tobacco chewing and in 8 patients (8%) history of smoking was obtained.

Table 7. Body mass index (BMI)

Body mass index	Distribution (n=100)	
	Number	Percentage
18.5 to 22.99	1	1.00
23.0 to 24.99	14	14.00
25.0 to 29.99	76	76.00
30 or more	9	9.00
Total	**100**	**100.00**



We observed in 76 patients (76%) BMI of 25 to 29.99, in 14 patients (14%) BMI of 23 to 24.99, in 9 patients (9%) BMI was >30 and in only 1 patient(1%) BMI was 18.5 to 22.99.The mean BMI was 27.27 ± 1.97 Kg/m^2.

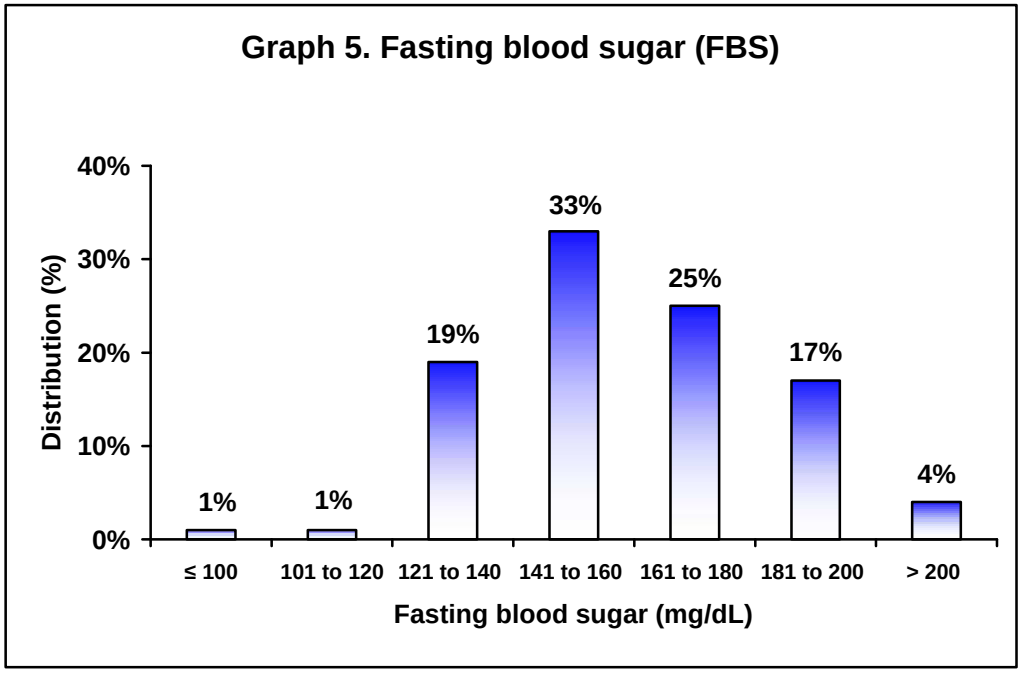
Table 8. Clinical Presentation / Observations and Complications

Clinical Presentation / Observations and Complications		Distribution (n=100)	
		No	**%**
Presentation/ Observations	Classical symptoms of DM (Polyphagia, polyuria, polydipsia)	86	86%
	Cerebrovascular accident	6	6.00
	Ischaemic heart disease	4	4.00
	Peripheral neuropathy	4	4.00
Complications	Diabetic retinopathy	21	21.00

6 patients (6%) presented with cerebrovascular accidents, 4 patients (4%) presented with ischaemic heart disease, 4 patients (4%) presented with peripheral neuropathy. Remaining 86 patients (86%) presented with one or the other symptom of diabetes. In 21 patients (21%) complication of diabetes that is retinopathy was observed.

Table 9. Fasting blood sugar (FBS)

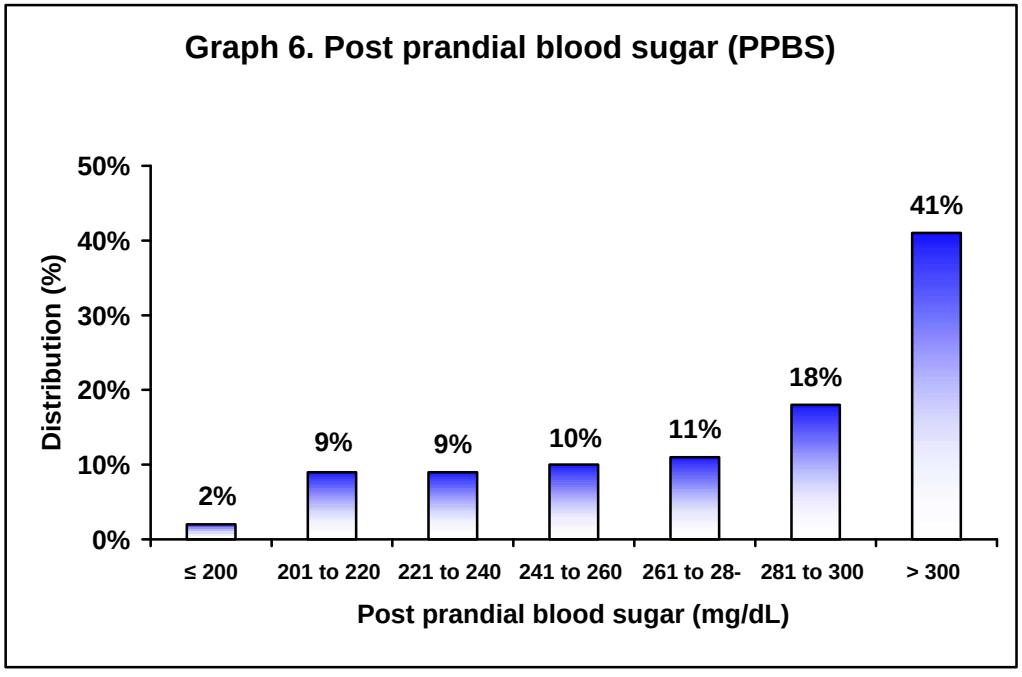
Fasting blood sugar (mg/dL)	Distribution (n=100)	
	Number	Percentage
≤ 100	1	1.00
101 to 120	1	1.00
121 to 140	19	19.00
141 to 160	33	33.00
161 to 180	25	25.00
181 to 200	17	17.00
> 200	4	4.00
Total	**100**	**100.00**



Most of our patients who presented had fasting glucose abnormality (98%), except 2 patients (2%) 1 (1%) had below 100 mg% and other (1%) had below 110 mg%.

Table 10. Post prandial blood sugar (PPBS)

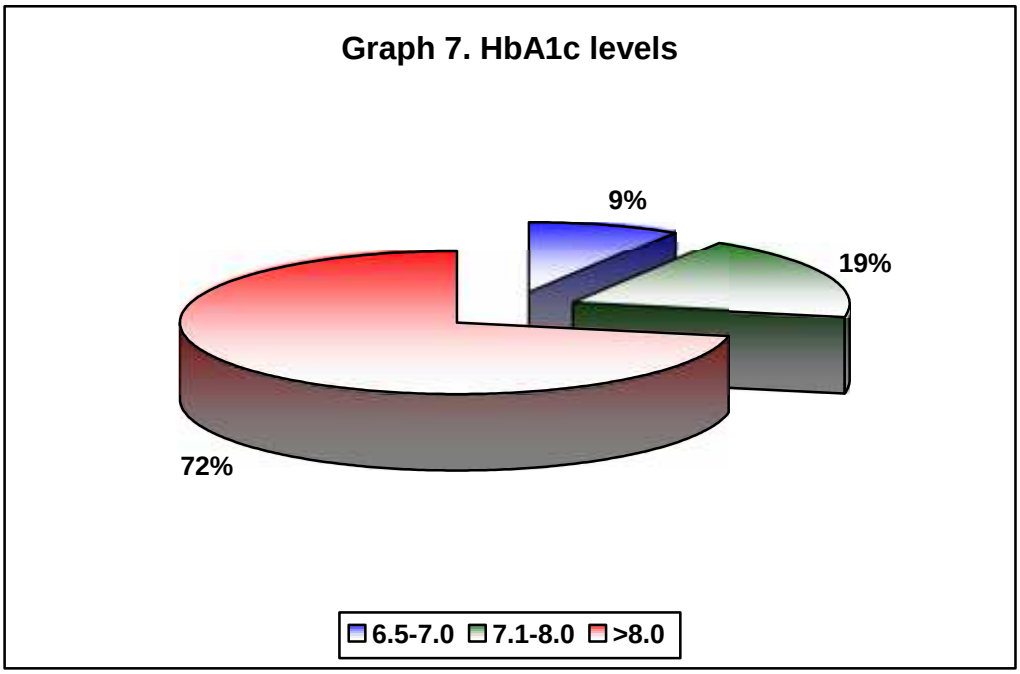
Post prandial blood sugar (mg/dL)	Distribution (n=100)	
	Number	Percentage
≤ 200	2	2.00
201 to 220	9	9.00
221 to 240	9	9.00
241 to 260	10	10.00
261 to 280	11	11.00
281 to 300	18	18.00
> 300	41	41.00
Total	**100**	**100.00**



Almost all our patients had post-prandial glucose abnormality (98%). 2 patients (2%) had post-prandial blood sugar <200 mg%, 1 patient (1%) had 132 mg% and other (1%) 194 mg%.

Table 11. HbA1c levels

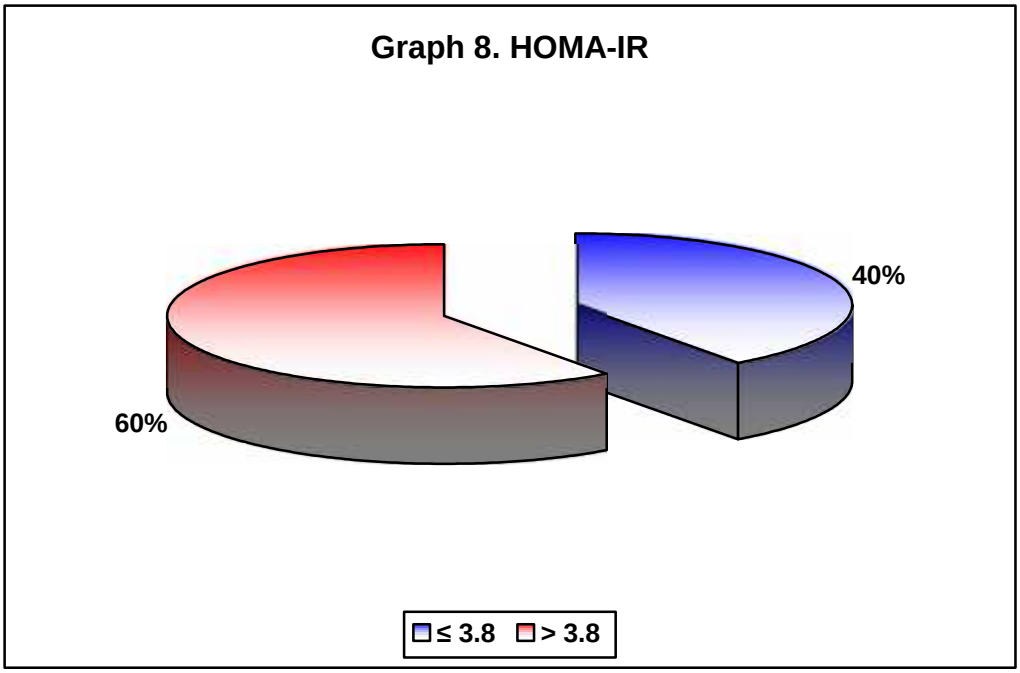
HbA1c (%)	Distribution (n=100)	
	Number	**Percentage**
6.5-7.0	9	9.00
7.1-8.0	19	19.00
>8.0	72	72.00
Total	**100**	**100.00**



In our present study, 72 patients (72%) had HbA1c of more than 8.0%, 19 patients (19%) had HbA1c between 7.1 to 8.0% and only 9 patients (9%) were between 6.5 to 7.0%. The mean HbA1c level was 9.61 ± 2.30%.

Table 12. HOMA-IR

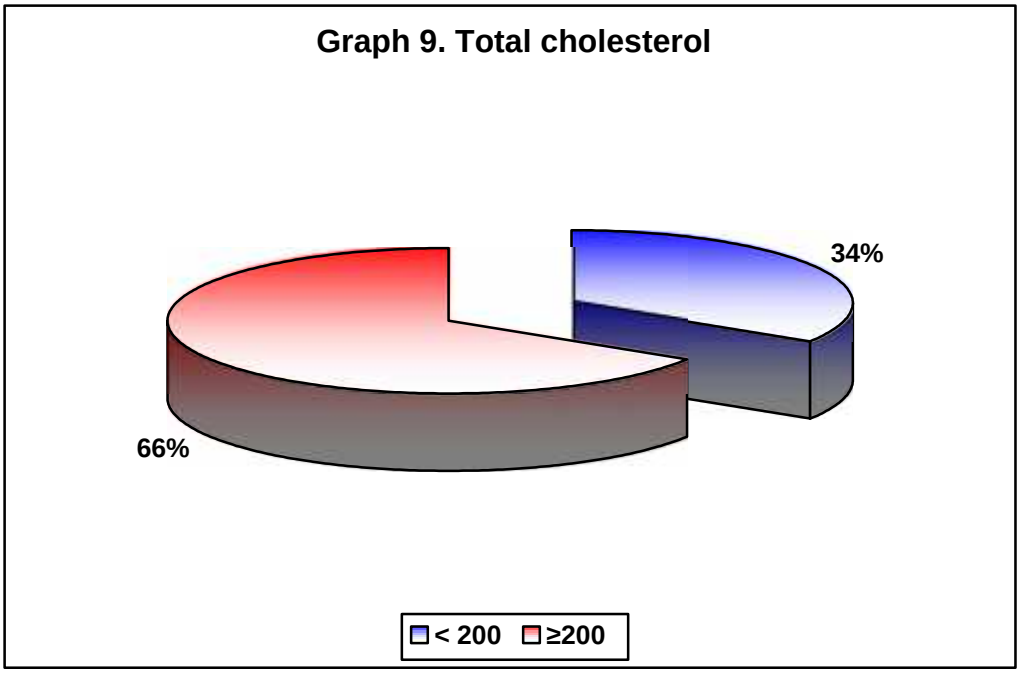
HOMA-IR	Distribution (n=100)	
	Number	**Percentage**
≤ 3.8	40	40.00
> 3.8	60	60.00
Total	**100**	**100.00**



40 patients (40%) had HOMA-IR of ≤3.8, remaining 60 patients (60%) had >3.8.

Table 13. Fasting lipids - Total cholesterol

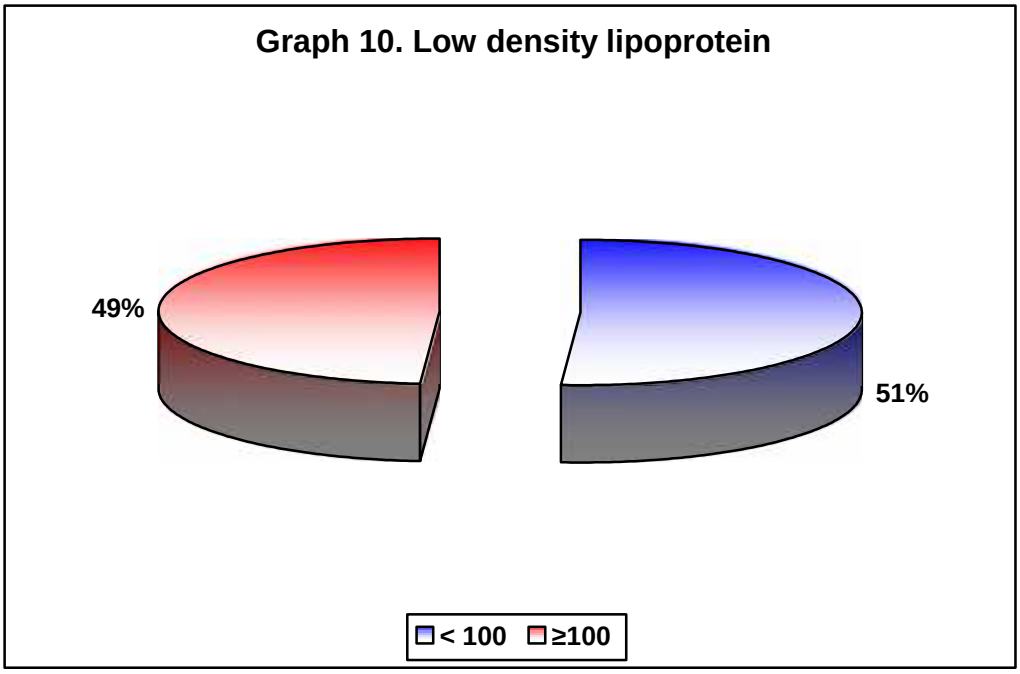
Total cholesterol (mg/dL)	Distribution (n=100)	
	Number	Percentage
<200 mg/dL	34	34.00
≥200 mg/dL	66	66.00
Total	**100**	**100.00**



34 patients (34%) had cholesterol <200 mg/dL and remaining 66 patients (66%) had >200 mg/dL.

Table 14. Fasting lipids - Low density lipoprotein (LDL)

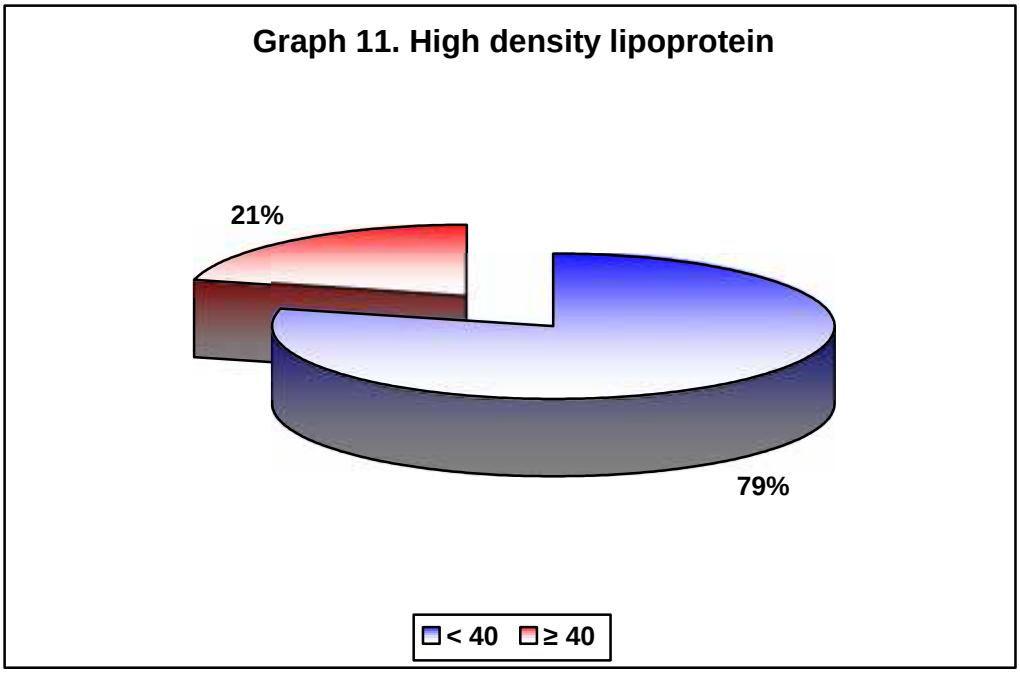
Low density lipoprotein (mg/dL)	Distribution (n=100)	
	Number	**Percentage**
< 100 mg/dL	51	51.00
≥100 mg/dL	49	49.00
Total	**100**	**100.00**



<100 mg/dL was observed in 51 patients (51%) and in 49 patients (49%) ≥100 mg/dL.

Table 15. Fasting lipids - High density lipoprotein (HDL)

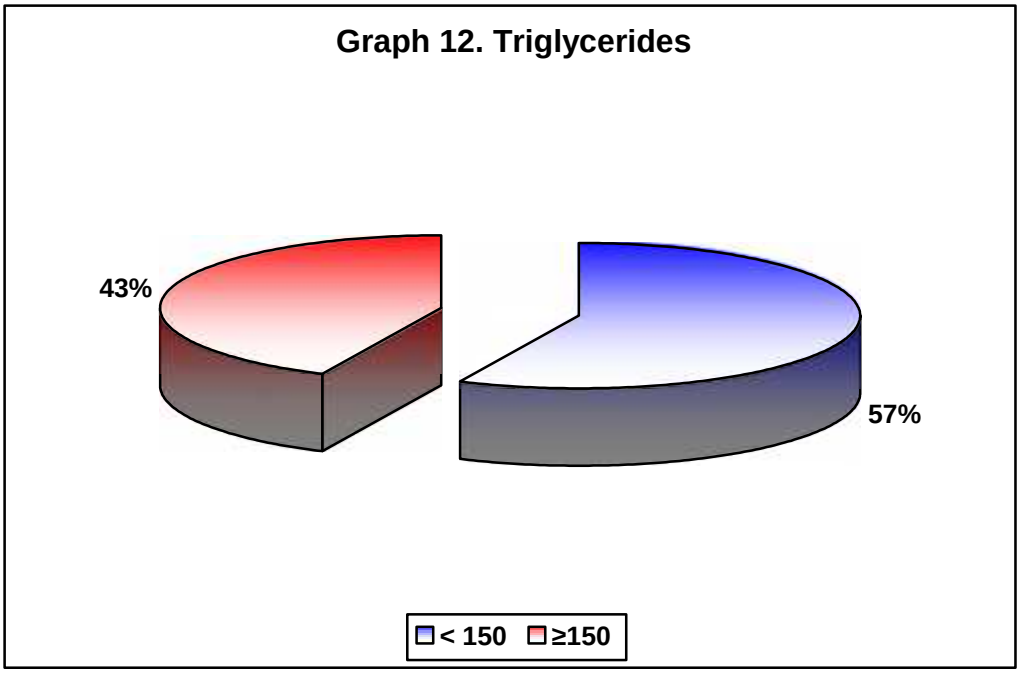
High density lipoprotein (mg/dL)	Distribution (n=100)	
	Number	**Percentage**
< 40 mg/dL	79	79.00
≥ 40 mg/dL	21	21.00
Total	**100**	**100.00**



In majority of patients (79%) HDL was <40 mg/dL, in 21 patients (21%) it was >40 mg/dL.

Table 16. Fasting lipids - Triglycerides

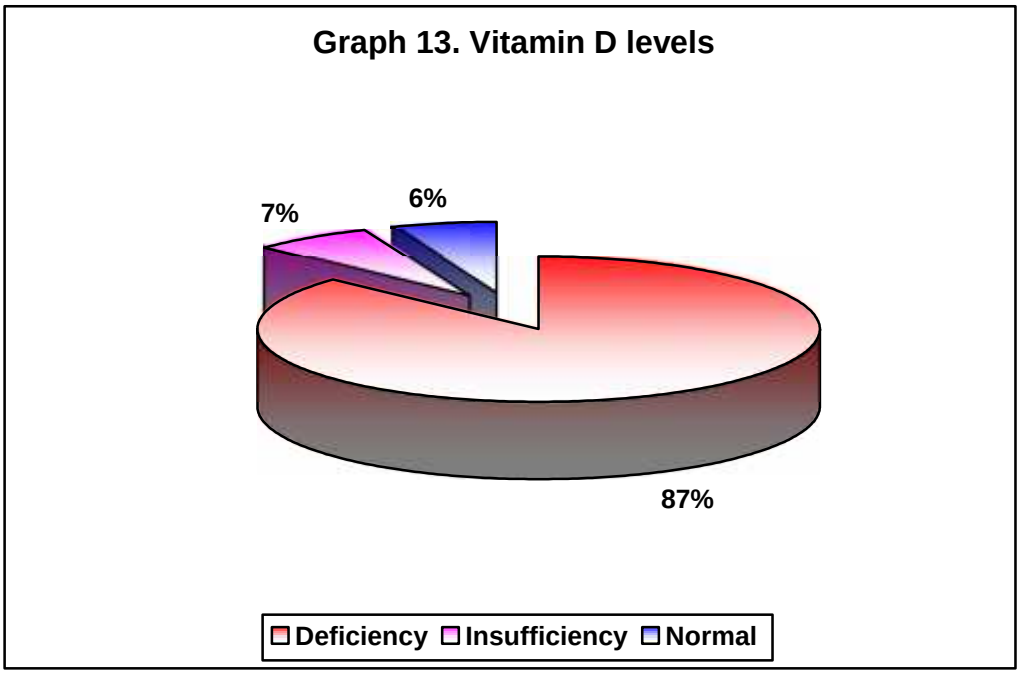
Triglycerides (mg/dL)	Distribution (n=100)	
	Number	**Percentage**
<150 mg/dL	57	57.00
≥150 mg/dL	43	43.00
Total	**100**	**100.00**



In 57 patients (57%) Triglycerides were <150 mg% and in remainder 43 patients (43%) it was >150 mg%.

Table 17. Vitamin D levels

Vitamin D (nmols/L)	Distribution (n=100)	
	Number	**Percentage**
Deficiency (< 50)	87	87.00
Insufficiency (50 to 75)	7	7.00
Normal (> 75)	6	6.00
Total	**100**	**100.00**



We observed in more than 87 patients (87%) the levels of Vitamin-D were below normal, in 7 patients (7%) the levels were insufficient and in remaining 6 patients (6%) the levels were either normal or more than normal.

Table 18. Correlation of Vitamin D levels with age

Age (years)	Vitamin D levels						Total (n=100)	
	< 50		≥ 50 to 75		> 75			
	No	%	No	%	No	%	No	%
≤ 40 to 50	15	78.95	3	15.79	1	5.26	19	100.00
51 to 60	28	87.50	3	9.38	1	3.13	32	100.00
61 to 70	28	96.55	0	0.00	1	3.45	29	100.00
71 to ≥ 80	16	80.00	1	5.00	3	15.00	20	100.00
Total	**87**	**87.00**	**7**	**7.00**	**6**	**6.00**	**100**	**100.00**

p=0.202

We observed the levels of Vitamin-D were less in age group, 6^{th} to 7^{th} decade 56%, in $>8^{th}$ decade 16% and $<4^{th}$ to 5^{th} decade 15%. In insufficient group there were 7 patients (<40 to 50 – 3; 51 to 60 – 3; 71 to >80 – 1). Patients who had either normal or more than normal levels were 6% (71 to >80 – 3; 61 to 70 – 1; 51 to 60 – 1; <40 to 50 – 1) (p-value = 0.202, is insignificant).

Table 19. Correlation of Vitamin D levels with Gender

Sex	Vitamin D levels						Total	
	< 50		≥ 50 to 75		> 75			
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**
Male	57	91.94	2	3.23	3	4.84	62	100.00
Female	30	78.95	5	13.16	3	7.89	38	100.00
Total	**87**	**87.00**	**7**	**7.00**	**6**	**6.00**	**100**	**100.00**

p = 0.126

Correlation of Vitamin-D levels with gender, we found that total of 87 patients (87%) had levels below normal (M-57; F-30). 7 (7%) were having insufficiency (M-02; F-05). And in only 6 patients (6%) we found levels were either normal or more than normal (M-03; F-03) (p-value = 0.126, is insignificant).

Table 20. Correlation of Vitamin D levels with Duration of Diabetes

Duration of diabetes (years)	Vitamin D levels						Total	
	< 50		≥ 50 to 75		> 75			
	No	%	No	%	No	%	No	%
Upto 5	27	84.38	4	12.50	1	3.13	32	100.00
6 to 10	32	96.97	1	3.03	0	0.00	33	100.00
11 to 15	13	81.25	1	6.25	2	12.50	16	100.00
> 15	15	78.95	1	5.26	3	15.79	19	100.00
Total	**87**	**87.00**	**7**	**7.00**	**6**	**6.00**	**100**	**100.00**

p=0.152

In patients with varying duration of diabetes mellitus ranging from <5 years to >15 years, 87 patients (87%) fell into below normal levels of Vitamin-D. 7 patients (7%) were in insufficient group. 6 patients (6%) it was observed the levels were either normal or more than normal, of these 6 patients (6%), 2 patients (12.50%) the duration of diabetes was 14 years and other 3 patients (15.79%) >15 years (p-value = 0.152, is insignificant).

Table 21. Correlation of Vitamin D levels with Body Mass Index (BMI)

Body Mass Index (kg/m^2)	Vitamin D levels						Total	
	< 50		≥ 50 to 75		> 75			
	No	%	No	%	No	%	No	%
18.5 to 22.99	1	100.00	0	0.00	0	0.00	1	100.00
23.00 to 24.99	12	85.71	2	14.29	0	0.00	14	100.00
25.00 to 29.99	66	86.84	4	5.26	6	7.89	76	100.00
30 or more	8	88.89	1	11.11	0	0.00	9	100.00
Total	**87**	**87.00**	**7**	**7.00**	**6**	**6.00**	**100**	**100.00**

p = 0.731

Attempt was made to correlate Vitamin-D levels with BMI, we observed in 1 patient (100%) the levels were below normal though BMI was almost normal. Remaining 86 patients (86%) the levels of Vitamin-D were below normal, as well they had BMI abnormality. Patients falling in insufficient group, all patients (7%) had abnormality of BMI. However in 6 patients (6%) though they had BMI abnormalities their levels were either normal or more than normal (p-value = 0.731, is insignificant).

Table 22. Correlation of Vitamin D levels with Fasting Blood Sugar

FBS	Vitamin D levels						Total	
	< 50		≥ 50 to 75		> 75			
	No	%	No	%	No	%	No	%
≤ 120 to 140	14	66.67	4	19.05	3	14.29	21	100.00
141 to 160	29	87.88	2	6.06	2	6.06	33	100.00
161 to 180	23	92.00	1	4.00	1	4.00	25	100.00
181 to 200	17	100.00	0	0.00	0	0.00	17	100.00
> 200	4	100.00	0	0.00	0	0.00	4	100.00
Total	**87**	**87.00**	**7**	**7.00**	**6**	**6.00**	**100**	**100.00**

p = 0.175

When levels were compared with fasting blood sugar most of patients with fasting glucose abnormality had low levels of Vitamin-D (87%). 7 patients (7%) had insufficient levels and only 6 patients (6%) had either normal or more than normal levels (p-value = 0.175, is insignificant).

Table 23. Correlation of Vitamin D levels with Post-Prandial Blood Sugar

PPBS	Vitamin D levels						Total	
	< 50		≥ 50 to 75		> 75			
	No	%	No	%	No	%	No	%
≤ 220 to 240	12	60.00	5	25.00	3	15.00	20	100.00
241 to 260	9	90.00	1	10.00	0	0.00	10	100.00
261 to 280	10	90.91	0	0.00	1	9.09	11	100.00
281 to 300	17	94.44	0	0.00	1	5.56	18	100.00
> 300	39	95.12	1	2.44	1	2.44	41	100.00
Total	**87**	**87.00**	**7**	**7.00**	**6**	**6.00**	**100**	**100.00**

p = 0.030

When levels were compared with post-prandial blood sugar most of patients with post-prandial glucose abnormality had low levels of Vitamin-D (87%). 7 patients (7%) had insufficient levels and only 6 patients (6%) had either normal or more than normal levels (p-value = 0.030, is significant).

Table 24. Correlation of Vitamin D levels with HbA1c

HbA1c (%)	Vitamin D levels						Total	
	< 50		≥ 50 to 75		> 75			
	No	%	No	%	No	%	No	%
6.5-7.0	6	66.67	2	22.22	1	11.11	9	100.00
7.1-8.0	13	68.42	3	15.79	3	15.79	19	100.00
>8.0	68	94.44	2	2.78	2	2.78	72	100.00
Total	**87**	**87.00**	**7**	**7.00**	**6**	**6.00**	**100**	**100.00**

p = 0.010

When levels were correlated with HbA1c, 6 patients (66.67%) had HbA1c between 6.5 to 7.0%. In 13 patients (68.42%) HbA1c was >7.0 and <8.0%. And remaining 68 patients (94.44%) HbA1c was >8.0%, all these patients had levels of Vitamin-D below normal.

In 2 patients (22.22%) HbA1c was 6.5 to 7.0%, in 3 patients (15.79%) 7.1 to 8.0% and in 2 patients (2.78%) it was >8.0%. In these 7 patients (7%) the levels were insufficient.

In remainder 6 patients (6%), 1 patient (11.11%) had HbA1c between 6.5 to 7.0%, 3 (15.79%) had 7.1 to 8.0% and 2 (2.78%) had >8.0%. In all these patients Vitamin-D levels were either normal or more than normal (p-value = 0.010, is significant).

Table 25. Correlation of Vitamin D levels with HOMA-IR

HOMA-IR	Vitamin D levels						Total	
	< 50		≥ 50 to 75		> 75			
	No	%	No	%	No	%	No	%
≤3.8	31	77.50	6	15.00	3	7.50	40	100.00
> 3.8	56	93.33	1	1.67	3	5.00	60	100.00
Total	**87**	**87.00**	**7**	**7.00**	**6**	**6.00**	**100**	**100.00**

p = 0.030

Comparison with HOMA-IR, in 31 patients (77.50%) the HOMA-IR was <3.8 and in 56 patients (93.33%) it was >3.8. In both the groups, levels of Vitamin-D were below normal.

6 patients (15.0%) had HOMA-IR <3.8, 1 (1.67%) had >3.8, all had insufficient Vitamin-D levels.

6 patients (6%) who had either normal or more than normal levels of Vitamin-D, 3 (7.50%) were having HOMA-IR of <3.8 (3%) and remaining 3 (5.0%) >3.8 (p-value = 0.030, is significant).

Table 26. Correlation of Vitamin D levels with Total Cholesterol

Cholesterol	Vitamin D levels						Total	
	< 50		≥ 50 to 75		> 75			
	No	%	No	%	No	%	No	%
<200	25	73.53	5	14.71	4	11.76	34	100.00
≥200	62	93.94	2	3.03	2	3.03	66	100.00
Total	**87**	**87.00**	**7**	**7.00**	**6**	**6.00**	**100**	**100.00**

p = 0.016

Out of 87 patients (87%), 25 (73.53%) had <200 mg% cholesterol levels and 62 (93.94%) had >200 mg%. All had levels of Vitamin-D less than normal.

7 patients (7%) were having insufficient levels of Vitamin-D, their cholesterol in 5 patients (14.71%) was <200 mg% and in 2 patients (3.03%) >200 mg%.

6 patients (6%) their levels of Vitamin-D was either normal or more than normal. 4 (11.76%) had <200 mg% cholesterol and 2 (3.03%) had >200 mg% (p-value = 0.016, is significant).

Table 27. Correlation of Vitamin D levels with Low Density Lipoprotein

LDL	Vitamin D levels						Total	
	< 50		≥ 50 to 75		> 75			
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**
< 100	43	84.31	2	3.92	6	11.76	51	100.00
≥ 100	44	89.80	5	10.20	0	0.00	49	100.00
Total	**87**	**87.00**	**7**	**7.00**	**6**	**6.00**	**100**	**100.00**

p = 0.027

87 patients (87%) had levels of Vitamin-D less than normal. In 43 (84.31%) LDL was <100mg% and remaining 44 (89.80%) >100mg%

7 (7%) had insufficient levels of Vitamin-D, their LDL in 2 patients (3.92%) <100mg% and 5 patients (10.20%) >100mg%.

All patients having either normal or more than normal levels of Vitamin-D i.e., 6 patients (11.76%) had LDL levels <100mg% (p-value = 0.027, is significant).

Table 28. Correlation of Vitamin D levels with High Density Lipoprotein

HDL	Vitamin D levels						Total	
	< 50		≥ 50 to 75		> 75			
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**
< 40	68	86.08	6	7.59	5	6.33	79	100.00
≥ 40	19	90.48	1	4.76	1	4.76	21	100.00
Total	**87**	**87.00**	**7**	**7.00**	**6**	**6.00**	**100**	**100.00**

p = 0.863

In 87 patients (87%) Vitamin-D levels were below normal, 68 patients (86.08%) had HDL <40mg% and 19 (90.48%) had >40mg%.

In insufficient group of Vitamin-D levels, 6 (7.59%) had <40mg% and only 1 (4.76%) had >40mg%.

Remaining 6 patients (6%) levels of Vitamin-D was either normal or more than normal, 5 patients (6.33%) had HDL levels <40mg% and 1 (4.76%) >40mg% (p-value = 0.863, is insignificant).

Table 29. Correlation of Vitamin D levels with Triglycerides

Triglycerides	Vitamin D levels						Total	
	< 50		≥ 50 to 75		> 75			
	No	%	No	%	No	%	No	%
< 150	49	85.96	3	5.26	5	8.77	57	100.00
≥ 150	38	88.37	4	9.30	1	2.32	43	100.00
Total	**87**	**87.00**	**7**	**7.00**	**6**	**6.00**	**100**	**100.00**

p = 0.319

In patients with below normal levels of Vitamin-D i.e., 87 patients (87%), 49 patients (85.96%) had triglycerides of <150mg% and in remaining 38 patients (88.37%) it was >150mg%.

7 patients (7%) who had insufficient levels of Vitamin-D, 3 (5.26%) were having triglycerides levels of <150mg% and 4 (9.30%) had >150mg%.

Remaining 6 patients (6%), levels of Vitamin-D was either normal or more than normal. 5 (8.77%) had <150mg% triglycerides and only 1 (2.32%) >150mg% (p-value = 0.319, is insignificant).

Chapter 6

Discussion

DISCUSSION

In the present study of 100 patients with type 2 diabetes mellitus, we observed the levels of Vitamin-D and compared it to various factors.

In our study, patients age ranged from 39 to 85 years. 49 patients were in the age group of >60 years. 44 patients had low levels of Vitamin-D, 1 had insufficient levels and 4 had either normal or more than normal levels. Observation was levels of Vitamin-D were low with increasing age (p=0.202; insignificant). This observation is similar to a study by Baynes KCR et al[98] (p=<0.05; significant). May be this finding in their study is because of adjusting various confounding factors and following up of these patients for nearly 30 years.

One study by Dalgard C et al[99] observed the low levels with increasing age. The reason for this could be the synthesis of Vitamin-D decreases with increasing age due to reduced concentration of 7-dehydrocholesterol in the skin and may be due to reduced absorption of oral Vitamin-D.

Similarly taking gender into consideration, no significant difference was found (p=0.126; insignificant) in our study. Same observation was by Ford ES et al.[100]

In our study, the duration of diabetes varied from <5 to >15 yrs. 87 patients (87%) having low levels, 28 patients had duration of >10 years and 59 patients had duration of <10 years. In insufficient group; only 2 patients >10 years and 5 patients <10 years. In 5 patients though duration was >10 years their

levels of Vitamin-D were either normal or more than normal (p=0.152; insignificant). This is in sharp contrast, study by Braun TR et al[101] who observed relationship between duration of diabetes.

All our patients were on treatment either with oral hypoglycaemic agents (64%) or insulin preparations (36%). All of these patients when presented had either fasting blood sugar or post-prandial blood sugar abnormality reflecting poor diabetic status. Most of our patients were having low or insufficient levels of Vitamin-D (except 6 patients who had levels either normal or more than normal). Same conclusion was drawn by Braun TR et al.[101] This issue needs further evaluation comparing the diabetic patients with healthy non-diabetic individuals. And this could be reason for low levels of Vitamin-D, who had both fasting and post-prandial blood sugar abnormality.

In our study 10 patients (10%) had associated hypertension and 2 patients (2%) had cerebrovascular accident. In a study by Braun TR et al[101] found the low levels of Vitamin-D in patients with associated hypertension. Our study, we found 7 patients (7%) had low levels of Vitamin-D and 3 patients (3%) had insufficient levels who were hypertensives. Another study by Shankar A et al[90] in pre-diabetics with associated hypertension the levels of Vitamin-D were low. In 2 patients with old cerebrovascular accident the levels of Vitamin-D were less. Whether it was because of associated cerebrovascular accident or type 2 diabetes mellitus difficult to state.

We obtained in 14 patients (14%) history of alcohol consumption, 9 (9%) tobacco chewing, 8 patients (8%) smoking history. Whether these confounding

factors have effects on Vitamin-D levels is not clear. Similar observation was found by Braun TR et al.[101] Study by Baynes KCR et al[98] found no correlation with alcohol consumption or smoking.

When levels of Vitamin-D were compared with BMI, the levels were less in patients with BMI above 23.0, however, p-value was not statistically significant (p=0.731). In a study by Braun TR et al[101] correlation between BMI and low levels of Vitamin-D had significant p-value (p=<0.0001). This difference could be because of small sample size (100 patients) in our study compared to their sample size of 887 patients. This is in sharp contrast; study by Baynes KCR et al[98] who observed no correlation with BMI.

Significant proportion of patients (86%) presented with symptoms pertaining to diabetes, 6 patients (6%) with cerebrovascular accident, 4 (4%) with ischaemic heart disease and 4 (4%) with peripheral neuropathy. We observed 21 patients (21%) though did not have visual disturbances on presentation, had either mild or moderate diabetic retinopathy.

When levels were compared with fasting blood sugar, most of patients with fasting glucose abnormality had low levels 87%, 7% had insufficient levels and 6% had either normal or more than normal levels (p=0.175; insignificant). Braun TR et al[101] in their study (sample size 887 cases), observed low levels of Vitamin-D are associated with fasting glucose abnormality (p=0.022; significant). Similarly study done by Shanthi B et al[102] (sample size 50 patients) found a negative correlation of Vitamin-D with fasting glucose abnormality (p-0.534; insignificant).

Study by Lu L et al[103] and Kotwal SK et al[104] found low levels of Vitamin-D when compared with increasing fasting blood sugars.

When levels were compared with post-prandial blood sugar, almost all our patients with post-prandial blood sugar abnormality had either low or insufficient levels (p=0.030; significant). Same was observed by Shanthi B et al[102] (p=0.511; insignificant).

When correlated with HbA1c the levels were definitely affected with HbA1c > 6.5 and above. 6 patients (6%) though they had HbA1c abnormality still they had either normal or more than normal Vitamin-D levels, p-value being significant (p=0.010). Similar observation was made by Dalgard C et al[99] (p=0.01; significant).

In another study by Shanthi B et al,[102] though they had low levels of Vitamin-D compared with HbA1c, p-value was non-significant(sample size -50; p-0.229).

In a study done by Lu L et al,[103] they found an inverse association between Vitamin-D levels and HbA1c, p-value was significant (p=<0.0001).

We observed in patients with HOMA-IR <3.8 and in patient >3.8, in both these groups levels of Vitamin-D were low. Similarly in patients with insufficient levels of Vitamin-D (7 patients - 6 < 3.8; 1 > 3.8). HOMA-IR in remaining 6 patients (3 patients < 3.8; 3 patients >3.8), the levels were either normal or more than normal, p-value being statistically significant (p=0.030). Same observation was by Lu L et al[103] and Dalgard C et al.[99]

An attempt was made to correlate Vitamin-D levels with fasting lipids we found following associations:

There was abnormality of Vitamin-D levels compared with cholesterol levels. In both the groups of patients with normal or abnormal levels of cholesterol, Vitamin-D was low (87%). In 6 patients (6%) levels of Vitamin-D were either normal or more than normal (4 patients the cholesterol levels were < 200 mg% and in 2 patients > 200 mg%). In remaining 7 patients (7%) the levels of Vitamin-D were insufficient (5 patients had cholesterol levels <200mg% and 2 patients had >200mg%); p= 0.016; significant.

Comparison with LDL levels, patients with normal as well as elevated levels (LDL), significant proportion of patients 87% had low levels of Vitamin-D. In remaining 7 patients (7%) it was insufficient levels (2 had LDL < 100 mg% and 5 had >100 mg%). And 6 patients (6%) it was either normal or more than normal levels (all had LDL levels < 100 mg%); p=0.027; significant.

Similar observation was made when compared with HDL levels. Patients with HDL levels of < 40 mg% and HDL levels of > 40 mg%, in both the groups of patients (87%) the levels of Vitamin-D were below normal. Remaining 7 patients (7%) (6 had HDL < 40 mg% and 1 > 40 mg%) the levels were insufficient. And in 6 patients (6%) (5 were having HDL <40mg% and 1 > 40 mg%) levels were either normal or more than normal; p=0.863; insignificant.

Finally comparison of Vitamin-D levels with triglycerides, either with normal or elevated levels (<150 mg% or > 150 mg%) 87 patients (87%) had low levels of Vitamin-D, 7 patients (7%) had insufficient levels (3 patients

<150mg%; 4 patients >150mg%). In 6 patients (6%) the levels of Vitamin-D were either normal or more than normal, however in 5 patients the triglycerides levels were <150mg% and in only 1 patient >150mg%; p=0.315; insignificant. This is in sharp contrast, study by Braun TR et al[101] factors like cholesterol, high density lipoprotein, low density lipoprotein and triglycerides compared with levels of Vitamin-D did not have significant correlation.

One study by Lu L et al[103] found low levels of high density lipoprotein (HDL) was associated with low levels of Vitamin-D. Similar observation was by Ford ES et al.[100]

When an attempt was made to analyse the available data both qualitatively and quantitatively to find out correlation of levels of Vitamin-D in patients of type 2 diabetes mellitus we found a significant correlation when compared with post-prandial blood sugar, HbA1c, HOMA-IR, cholesterol and low density lipoprotein (LDL) (p value being significant). Other variables like age, gender, duration of diabetes, body mass index, fasting blood sugar, high density lipoprotein (HDL) and triglycerides when compared, did not show significant correlation. Reason could be because of our small sample size (100 patients).

To overcome this bias with confounding factors and co-morbid conditions may be large sample size is required. And also comparison of diabetic individuals with non-diabetic healthy individuals is essential to find out the true correlation of Vitamin-D levels.

Chapter 7

Conclusion

CONCLUSION

In present study of 100 patients with type 2 diabetes mellitus the levels of Vitamin-D when compared with factors like post-prandial blood sugar, HbA1c, HOMA-IR, cholesterol and low density lipoprotein (LDL), we found a significant correlation (p-value being statistically significant).

In patients with other variables like age, gender, duration of diabetes, body mass index, fasting blood sugar, high density lipoprotein (LDL) and triglycerides, we found no significant correlation (p-value being statistically insignificant).

Further studies to compare patients with type 2 diabetes mellitus and non-diabetic healthy individuals may be necessary to know the relationship with Vitamin-D levels.

We feel it is worth to study by adjusting the comorbid conditions/ confounding factors and comparing with levels of Vitamin-D, may have reflection whether relationship really exists.

Owing to our small sample size (100 patients), it is necessary large sample size may be required to overcome these bias.

Chapter 8

Summary

SUMMARY

In present study of 100 patients with type 2 diabetes mellitus admitted in Department of Medicine KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the period from January 2012 to December 2012, to find out correlation of Vitamin-D levels with various factors.

The results observed were a significant correlation of Vitamin-D levels with variable factors – post-prandial blood sugar, HbA1c, HOMA-IR, cholesterol and low density lipoprotein (LDL). However, we did not find significant correlation with variable factors – age, gender, duration of diabetes, body mass index, fasting blood sugar, high density lipoprotein (HDL) and triglycerides.

Chapter 9

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Annexures

Annexure I

ANNEXURE I – CONSENT FORM

"ESTIMATION OF LEVELS OF VITAMIN D IN TYPE 2 DIABETES MELLITUS - ONE YEAR CROSS-SECTIONAL STUDY"

Objective and purpose of the study:

. This research is intended to estimate the Serum Vitamin D levels in patients with Type 2 Diabetes Mellitus. The principal investigator of the study is Dr. **** ******** under the guidance of Dr. **** *******

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 same 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 a participant you may call Dr. **** ******, Principal and Chairman, J.N.M.C Ethical Committee for Human Research phone number **** ******.

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 authorized representative/ Guardian	: …….
Signature/ Left Thumb Impression.	: …….
Witness's Name	: …….
Signature/ Left Thumb Impression.	:…….
Investigators name and Signature	: …….
Date and Place	: …….

Dr. ***** *********
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Ph.No. ********* Ext. ******

Dr. ***** *******
Post-Graduate,
Department of Medicine, J. N.
Medical College, Belgaum 10.
Ph.No. ****** ***** Ext. *****

Annexures

Annexure II

ANNEXURE II – PROFORMA

Case No.: IP number:

Patient Name: ID number:

Age: Sex:

Address:

Occupation:

Date of admission:

PRESENTING SYMPTOMS

Classical symptoms of diabetes mellitus (Polyphagia, Polyuria, Polydipsia)	Yes / No
Ischaemic heart disease	Yes / No
Peripheral vascular disease	Yes / No
Cerebrovascular accident	Yes / No
Peripheral neuropathy	Yes / No
Retinopathy	Yes / No
Any other relevant history	Yes / No

HISTORY

Diabetic history

Duration:

Medication:

Hypertension	Yes / No
Ischaemic heart disease	Yes / No
Cerebrovascular disease	Yes / No
Family history	Yes / No

Personal history

Diet: Vegetarian / Mixed

Smoking: Yes / No

Tobacco chewing: Yes / No

Alcohol: Yes / No

Menstrual history:

General physical examination:

BMI :

Vitals :

Pulse rate:

Blood pressure: Systolic: Diastolic:

Respiratory rate:

Temperature: Febrile / Afebrile

Pallor Yes / No

Icterus: Yes / No

Oedema: Yes / No

Cyanosis: Yes / No

Clubbing: Yes / No

Lymphadenopathy: Yes / No

Systemic examination:

Respiratory system:

Cardiovascular system:

Per abdomen:

Central nervous system:

Fundoscopy

Laboratory investigations

Complete blood count

Haemoglobin (gm%):

Total count:

Differential count:

Platelet count:

ESR:

Red blood cell count:

Peripheral smear:

Renal profile

Urea:

Creatinine

Sodium

Potassium

Liver function

Total bilirubin

Direct bilirubin

Total protein

Serum albumin

SGOT

SGPT

Alkaline phosphatase

Urine

Routine

Microscopy

Ketone bodies

Blood sugar levels

Fasting:

Post prandial:

HbA1c:

Fasting insulin levels:

HOMA-IR:

Fasting Serum 25-hydroxy vitamin D:

Lipid profile:

Cholesterol

Low density lipoprotein

High density lipoprotein

Triglycerides

Annexures

Annexure III

ANNEXURE III – MASTER CHART

-	-	Absent
+	-	Present
AB	-	Absent
B	-	Business
Bpm	-	Beats per minute
CO	-	Cooli
DR	-	Diabetic retinopathy
F	-	Female
Fa	-	Farmer
HW	-	Housewife
INS	-	Insulin
Kg/m^2	-	Kilogram per meter square
LB	-	Labour
LH	-	Left hemiplegia
M	-	Male
m	-	Mentruating
mg/dL	-	Milligram per deci litre
mm Hg	-	Millimeter of mercury
MP	-	Menopause
MX	-	Mixed
N	-	Normal
OHA	-	Oral hypoglycaemic agents
PD	-	Polydipsia

PP	-	Polyphagia
PU	-	Polyuria
RH	-	Right hemiplegia
S	-	Service
V	-	Vegetarian