"SERUM MAGNESIUM LEVELS IN TYPE 2 DIABETES MELLITUS AND ITS ASSOCIATION WITH THE MICROVASCULAR COMPLICATIONS"

REG NO. BG0113010

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

APRIL - 2016

KLE UNIVERSITY, BELGAUM,

KARNATAKA

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LIST OF ABBREVIATIONS USED

α	-	Alpha
β	-	Beta
µg/min	-	Micro gram per minute
ACE	-	Angiotensin-converting enzyme
ADA	-	American Diabetes Association
ADH	-	Antidiuretic hormone
AGEs	-	Advanced glycosylated end products
ARBs	-	Antiotensin receptor blockers
ARIC	-	Atherosclerosis Risk in Communities
ATN	-	Acute tubular necrosis
ATP	-	Adenosine triphosphate
BMI	-	Body mass index
BP	-	Blood pressure
CAD	-	Coronary artery disease
cAMP	-	Cyclic adenine monophosphate
CT	-	Computed tomography
cTAL	-	Thick ascending loop
DCCT	-	Diabetes Control and Complications Trial
DCT	-	Distal convoluted tubule
dL	-	Decilitre
DM	-	Diabetes mellitus
DNA	-	Deoxyribonucleic acid
DPN	-	Diabetic peripheral neuropathy
DRI’s	-	Dietary reference intakes

e.g.	-	For example
ECF	-	Extracellular fluid
ESRD	-	End-stage renal disease
FDA	-	Food and Drug Administration
FPG	-	Fasting plasma glucose
g	-	Grams
GDM	-	Gestational diabetes mellitus
GFR	-	Glomerular filtration rate
gms	-	Grams
HbA1C	-	Glycosylated haemoglobin
HDL	-	High density lipoprotein
HDL-C	-	High-density lipoprotein-cholesterol
HNF	-	Hepatocyte nuclear transcription factor
Hz	-	Hertz
IDDM	-	Insulin dependent diabetes mellitus
IDF	-	International Diabetes Federation
ie,	-	That is
IFG	-	Impaired fasting glucose
IGT	-	Impaired glucose tolerance
IPF	-	Insulin promoter factor
IRMA	-	Intraretinal microvascular abnormality
kg	-	Kilograms
LDL	-	Low-density lipoprotein
LDL-C	-	Low-density lipoprotein-cholesterol
m	-	Meter

mEq	-	Milliequivalents
Mg	-	Magnesium
mg	-	Milligram
MI	-	Myocardial infarction
mmHg	-	Millimeter of mercury
mmol	-	Millimole
MODY	-	Maturity Onset Diabetes of Young
mol	-	Mole
n	-	Total number
NA	-	Not applicable
Na	-	Sodium
NIDDM	-	Non-insulin dependent diabetes mellitus
NPDR	-	Non Proliferative Diabetic Retinopathy
NVD	-	Neovascularisation disc
NVE	-	Neovascularisation elsewhere
p	-	Probability value
PAD	-	Peripheral arterial disease
PAI-1	-	Type-1 plasminogen activator inhibitor
PDR	-	Proliferative diabetic retinopathy
PT	-	Proximal tubules
PTH	-	Parathyroid hormone
RDA	-	Recommended Dietary Allowances
RNA	-	Ribonucleic acid
SD	-	Standard deviation
SIADH	-	Syndrome of inappropriate antidiuretic hormone

T2DM	-	Type 2 daibetes mellitus
TAH	-	Thick ascending limb of the loop of Henle
TGF	-	Transforming growth factor
TRPM6	-	Transient receptor potential channel melastatin 6
UKPDS	-	United Kingdom Prospective Diabetes Study
VEGF-A	-	Vascular endothelial growth factor A
vs	-	Versus
WHO	-	World Health Organization
Yrs	-	Years

ABSTRACT

Background and objectives

Hypomagnesemia has been associated with type 2 diabetic mellitus and is known to be a risk factor for microvascular complications. This study aimed to evaluate serum magnesium levels in patients with type 2 DM and correlate them with microvascular complications.

Methodology

The present one year hospital based cross-sectional study was done on 150 patients with type 2 diabetes mellitus from January 2014 to December 2014 in Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. Serum magnesium levels were assessed in all the diabetic patients and they were also tested for presence of microvascular complications.

Results

In the present study majority of the patients (71.33%) were males and male to female ratio was 2.48:1. The commonest age group was > 60 years (50%) and the mean age was 60.38 ± 10.81 years. The duration of diabetes in 45.33% of the patients was between 6 to 10 years and mean duration was 7.43 ± 4.11 years. Fasting blood sugar levels were ≥ 126 mg/dL in 77.33% of the patients and HbA1c levels were ≥8.5% in 51.33% of the patients. Serum magnesium levels were < 1.8 mg/dL in 41.33% of the patients. Microvascular complications were present in 54.67% of the patients and diabetic retinopathy was present in 32%. Diabetic nephropathy and diabetic peripheral neuropathy were seen in 36% of the

patients each. Hypomagnesemia was associated with microvascular complications including diabetic retinopathy, diabetic nephropathy and diabetic neuropathy ($p<0.050$). Also association was found between serum magnesium levels glycaemic control and duration of diabetes ($p<0.050$).

Conclusion

Hypomagnesemia is widely prevalent in patients with type 2 diabetes mellitus and a major risk factor for the development microvascular complications that is, diabetic retinopathy, nephropathy and neuropathy.

Keywords

Hypomagnesemia; Diabetic nephropathy; Diabetic neuropathy; Diabetic retinopathy; Microvascular complications ; Type 2 diabetes mellitus;

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INTRODUCTION

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. It results from defects in insulin secretion, insulin action or both. The effect of diabetes mellitus include long term damage, dysfunction and failure of various organs, eyes, kidneys, nerves and heart and blood vessels. Several distinct types of DM are caused by complex interaction of genetics and environmental factors. Depending on etiology of the DM factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production.[1]

Type 2 diabetes mellitus is a metabolic and endocrine disease characterised by hyperglycemia associated with insulin resistance and/or defective insulin secretion.[2] Type 2 Diabetes mellitus accounts for approximately 90-95% of all diagnosed cases of diabetes.[3]

The prevalence of diabetes is rapidly rising all over the globe at an alarming rate.[4] It is important to note that the rise in prevalence is seen in all six inhabited continents of the globe.[5] Over the past 30 years, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged people. Although there is an increase in the prevalence of type 1 diabetes also, the major driver of the epidemic is the more common form of diabetes, namely type 2 diabetes, which accounts for more than 90% of all diabetes cases.[6]

The epidemic of diabetes is most pronounced in India as the World Health Organization (WHO) reports show that 32 million people had diabetes in the year 2000.[5] 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.[7]

Diabetes mellitus leads to impaired metabolism of carbohydrates, proteins, fats, water and electrolytes. The persistence of these metabolic disturbances lead to permanent and irreversible functional and structural changes in the cells of the body which in turn lead to the development of "diabetic complications", characteristically affecting, the cardiovascular system, eye, kidney and nervous system mainly.[8] Chronic complications of diabetes mellitus can be divided into vascular and nonvascular complications. The vascular complications of DM are further subdivided into microvascular (retinopathy, neuropathy, nephropathy) and macrovascular complications [coronary artery disease (CAD), peripheral arterial disease (PAD), cerebrovascular disease].[1]

Micronutrients have been investigated as potential, preventive and therapeutic agents for type 2 diabetes mellitus and their complications.[9] In particular, diabetes has shown to be associated with abnormalities in the metabolism of zinc, chromium, copper, magnesium and manganese.[10] Out of these magnesium has been investigated as a clinically significant electrolyte, for a long term global policy to lower the burden of diabetes mellitus, with new findings and researches.[11] Studies have shown that magnesium levels are lower in patients with diabetes compared with nondiabetic controls.[12] The reported incidence of hypomagnesemia in patients with type 2 DM varies between 13.5 to as high as 47.7%.[13]

Magnesium is the fourth most abundant cation in the human body and the second most abundant intracellular cation.[14] It plays an important role in the carbohydrate metabolism. It serves as a cofactor for all enzymatic reactions that require kinases.[15] It is also an essential enzyme activator for neuromuscular excitability and cell permeability, a regulator of ion channels and mitochondrial function, a critical element in cellular proliferation and apoptosis, and an important factor in both cellular and humoral functions.[16] Magnesium depletion has a negative impact on glucose homeostasis and insulin sensitivity in patients with type 2 diabetes as well as on the evolution of complications such as retinopathy, arterial atherosclerosis and nephropathy. Moreover a low serum magnesium level is strong, independent predictor of development of microvascular complications in type 2 DM.[17]

Although, serum magnesium levels are known to be low in type 2 DM, the entity of hypomagnesemia very often remains under-diagnosed and under-evaluated due to its usual asymptomatic presentation. Also, to date, there are very few studies which have evaluated the association of serum magnesium levels with the microvascular complications especially in India. Hence, this study was planned to evaluate the serum magnesium levels in patients with type 2 DM and to correlate them with the microvascular complications.

OBJECTIVES

The objectives of the present study were to;

- To assess the serum magnesium levels in type 2 diabetes mellitus.
- To correlate the serum magnesium levels with the microvascular complications (diabetic retinopathy, nephropathy and neuropathy).

REVIEW OF LITERATURE

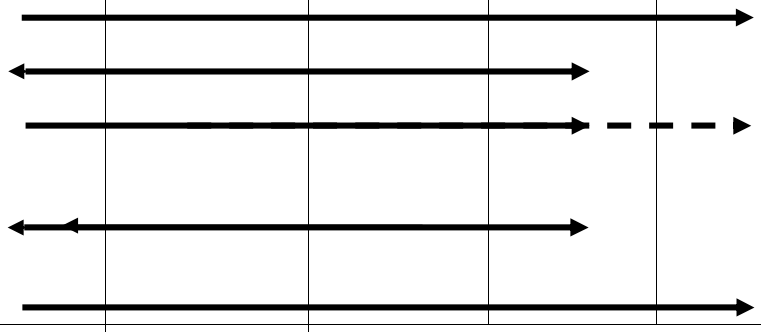
DIABETES MELLITUS

Diabetes mellitus is defined by a group of common metabolic disorders that share the phenotype of hyperglycemia. Various types of diabetes mellitus exist and are caused by a complex interaction of genetics, environmental factors, and different lifestyles. The factors that contribute to hyperglycemia 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]

CLASSIFICATION OF DIABETES AND OTHER CATEGORIES OF GLUCOSE REGULATION

Diabetes mellitus is classified on the basis of the pathogenic process of hyperglycemia.[1]

Spectrum of glucose homeostasis and diabetes mellitus[1]

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		

Diabetes mellitus is categorized into 2 broad varieties: 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 are responsible for the common phenotype of hyperglycemia in type 2 diabetes mellitus.[1]

There are two changes in 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 is now not in use. Second, age is no more a criteria for diagnosis in the newer classification system.[1]

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.[1]

TYPE 2 DIABETES MELLITUS

This form of diabetes, accounts for ~90-95% of those with diabetes, and encompasses those individuals, who have insulin resistance and thus have relative (rather than absolute) insulin deficiency.[1]

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. 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 type 2 diabetes increases with age, obesity, and lack of physical activity. It occurs more frequently in women with prior GDM (Gestational diabetes mellitus),[18] and individuals with hypertension or dyslipidemia. It is often associated with a strong genetic predisposition, more so than type 1 diabetes mellitus.[19]

Epidemiology

Worldwide

The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. There are 382 million people living with diabetes worldwide. The worldwide prevalence of diabetes in adults (aged 20-79 years) was estimated to be 135, 285 million in 1995 and 2010 respectively and is expected 300 million in 2025 and 439 million in 2030.[5,20-26]

Sex predilection

The prevalence of diabetes is higher in men than women.[20-26]

Mortality

In 2012 it resulted in 1.5 million deaths worldwide making it the 8th leading cause of death and more than 80% of diabetic deaths occurring in low and middle-income countries. More than 21 million live births were affected by diabetes during pregnancy and > 79,000 children developed type 1 diabetes in 2013.[20-26]

Indian scenario

According to The International Diabetes Federation (IDF) estimation, India will have increase in people living with diabetes up to 87.0 million by 2030 from 50.8 million (2010), making it the 'Diabetes Capital' of the world.[27-29]

Risk factors for Type 2 Diabetes Mellitus[27]

- Family history of diabetes (i.e. parent or sibling with type 2 diabetes)

- Obesity (BMI≥25 kg/m2)
- Habitual physical inactivity
- Race/ethnicity (e.g. African, American, Hispanic American, Native American, Asian American, Pacific Islander)
- Previously identified IFG or IGT
- History of GDM or delivery of baby > 4kg (>9 Ib)
- Hypertension (blood pressure ≥ 140/90 mHg)
- HDL cholesterol level ≤ 35mg/dL (0.90mmol/L) and / or a triglyceride level ≥ 250mg/dL (2.82 mol/L)
- Polycystic ovary syndrome or acanthosis nigricans.
- History of vascular disease.

Symptoms

Symptoms are similar in both types of diabetes but vary in their intensity. Symptoms develop more rapidly in type 1 diabetes. The symptoms of diabetes include polyuria, polydipsia, polyphagia, weight loss, fatigue. People with diabetes have an increased risk of developing a number of serious health problems.[27]

Pathophysiology

Hyperglycemia results from lack of endogenous insulin, the deficiency of which, is either absolute, as in type 1 diabetes mellitus, or relative, as in type 2 diabetes mellitus. Relative insulin deficiency usually occurs because of resistance to the actions of insulin in muscle, fat, and the liver and an inadequate response by the pancreatic beta cell. Insulin resistance has been attributed to elevated levels of free

fatty acids in plasma,[30] and can lead to decreased glucose transport in muscle, elevated hepatic gluconeogenesis, and increased fat breakdown.

Presumably, the defects of type 2 diabetes mellitus occur when a sedentary lifestyle is superimposed on a susceptible genotype. The body mass index at which the risk for diabetes increases 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.[31] A simplified scheme for the pathophysiology of abnormal glucose metabolism in type 2 diabetes mellitus is depicted in the image below.

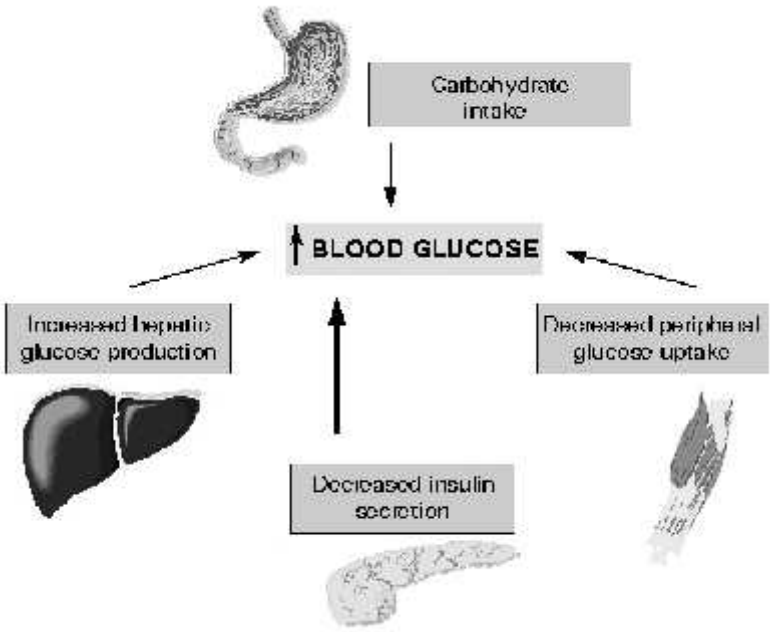


Figure 1. Pathophysiology of type 2 diabetes mellitus[32]

Hyperglycemia is known to be a major determinant of microvascular and metabolic complications. However, glycemia has a lesser effect on the macrovascular complications. Insulin resistance with associated abnormalities in

lipid metabolism (i.e., small dense low-density lipoprotein [LDL] particles, low high-density lipoprotein-cholesterol [HDL-C] levels, elevated triglyceride-rich remnant lipoproteins) and thrombotic (ie, elevated type-1 plasminogen activator inhibitor [PAI-1], elevated fibrinogen) abnormalities, as well as the conventional atherosclerotic risk factors (e.g., family history, smoking, hypertension, elevated low-density lipoprotein-cholesterol [LDL-C], low HDL-C), determine cardiovascular risk.[32]

Diagnosis

The National Diabetes Data Group and World Health Organization have issued diagnostic criteria for DM based on the following premises:[32]

***Criteria for the Diagnosis of Diabetes Mellitus*[33]**

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

[a]Random is defined as without regard to time since the last meal.
[b]Fasting is defined as no caloric intake for at least 8 h.
[c]The test should be performed using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water; not recommended for routine clinical use.

Complications

Uncontrolled diabetes can lead to an increased risk of heart disease, high blood pressure, stroke, neuropathy, renal failure, gum disease, blindness, foot and leg infections, sexual dysfunctions, pregnancy complications. It can also lead to

acute biochemical imbalances that cause life-threatening complications, such as diabetes ketoacidosis and hyperosmolar coma.[20,34]

Acute complications

- Diabetic ketoacidosis
- Hyperglycemic Hyperosmolar state
- Hypoglycemia

Chronic Complications [35-41]

The chronic complications of DM affect many organ systems and those may be responsible for the majority of morbidity and/or mortality associated with the disease.

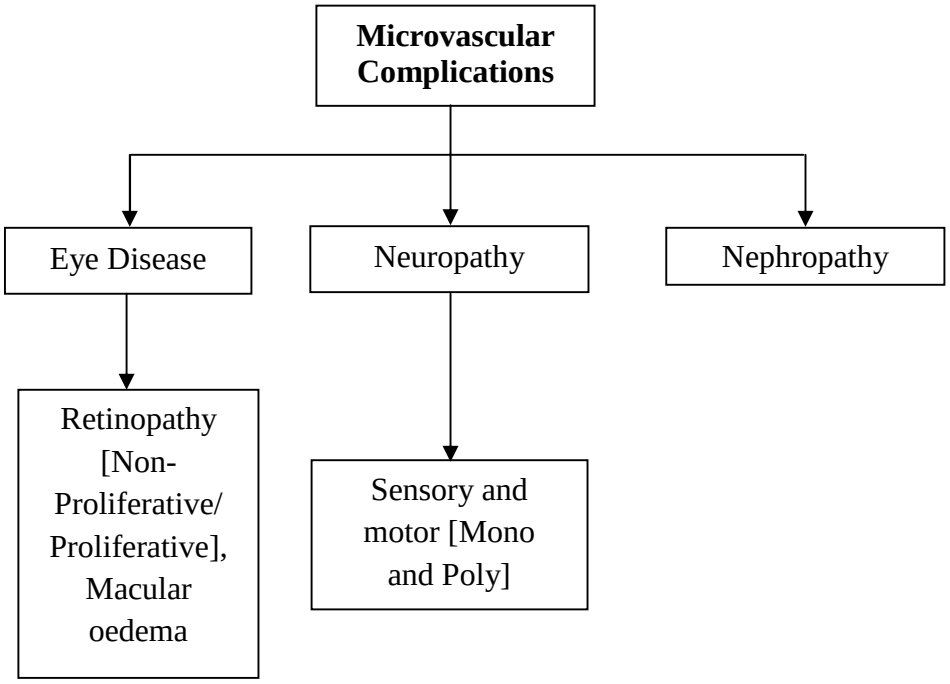


Figure 2. Microvascular complications seen in diabetes mellitus

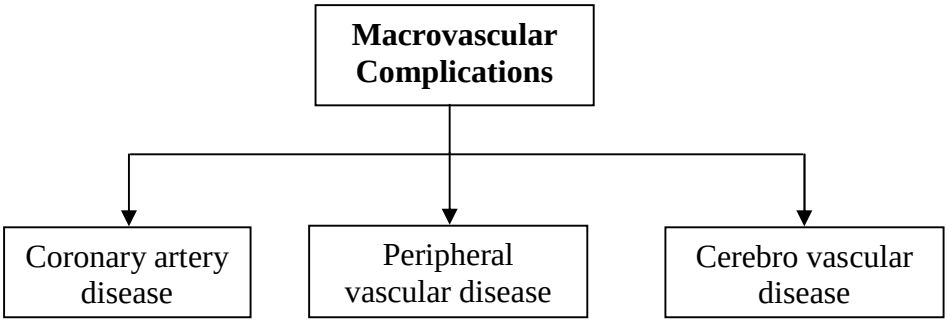


Figure 2. Macrovascular complications seen in diabetes mellitus

Other complications seen in diabetes mellitus:[35-41]

- Gastro-intestinal problems [Gastroparesis, diarrhea]
- Genitor-urinary problems [Uropathy / Sexual dysfunction]
- Dermatologic problems.
- Infections.
    - UTI
    - Tuberculosis
    - Candidiasis – oral / volvovaginal
    - Mucor mycosis
    - Necrotising fasciitis
    - Periodontitis
- Cataracts and Glaucomas.
- Duputrens contracture, Pseudogout

Mechanisms of Complications

Although chronic hyperglycemia is an important etiologic factor leading to complications of DM, the mechanism(s) by which it leads to such diverse cellular and organ dysfunction is unknown.[42]

Four theories have been proposed to explain the pathophysiology of chronic complications in DM.[1,32,43]

One theory is that increased intracellular glucose leads to the formation of advanced glycosylation end products (AGEs) via the nonenzymatic glycosylation of intra- and extracellular proteins. Nonenzymatic glycosylation results from the interaction of glucose with amino groups on proteins. AGEs are known to cross-link proteins (for example collagen, extracellular matrix proteins), accelerate atherosclerosis, promote glomerular dysfunction, reduce nitric oxide synthesis, induce endothelial dysfunction, and alter extracellular matrix composition and structure. The serum level of AGEs correlates with the level of glycemia, and these products accumulate as glomerular filtration rate declines.[44]

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

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

A fourth theory proposes that hyperglycemia increases the flux through the hexosamine pathway, which generates fructose-6-phosphate, a substrate for O-linked glycosylation and proteoglycan production. The hexosamine pathway causes glycosylation of proteins such as endothelial nitric oxide synthase and also regulates changes in gene expression of transforming growth factor B (TGF-B) or plasminogen activator inhibitor-1 (PAI-1).

Growth factors appear to play an important role in DM-related complications, and the above mentioned pathways increases their production. Vascular endothelial growth factor A (VEGF-A) is increased locally in diabetic proliferative retinopathy. TGF-B is increased in diabetic nephropathy and stimulates the production of collagen and fibronectin by mesangial cells. There is also a role of other growth factors, such as platelet-derived growth factor, epidermal growth factor, insulin-like growth factor I, growth hormone, basic fibroblast growth factor and insulin in DM-related complications. A unifying mechanism could be that hyperglycemia leads to increased production of reactive oxygen species in the mitochondria and these compounds may activate all four of the pathways. Hence, although hyperglycemia serves as the initial trigger for complications of diabetes, it is still unknown whether the same pathophysiologic processes are operative in all complications or whether some pathways predominate in certain organs.[45]

Glycemic Control and Complications

The Diabetes Control and Complications Trial (DCCT) provided definitive proof that reduction in chronic hyperglycemia can prevent many of the early complications of type 1 DM. This large multicenter clinical trial randomized over 1400 individuals with type 1 DM to either intensive or conventional diabetes management, and prospectively evaluated the development of retinopathy, nephropathy, and neuropathy. Individuals in the intensive diabetes management group received multiple administrations of insulin each day along with extensive educational, psychological, and medical support.[46]

Individuals in the conventional diabetes management group received twice-daily insulin injections and quarterly nutritional, educational, and clinical evaluation. The goal in the former group was normoglycemia; the goal in the latter group was prevention of symptoms of diabetes. Individuals in the intensive diabetes management group achieved a substantially lower hemoglobin A1C (7.3%) than individuals in the conventional diabetes management group (9.1%).[46]

The DCCT demonstrated that improvement of glycemic control reduced nonproliferative and proliferative retinopathy (47% reduction), microalbuminuria (39% reduction), clinical nephropathy (54% reduction), and neuropathy (60% reduction). Improved glycemic control also slowed the progression of early diabetic complications. There was a nonsignificant trend in reduction of macrovascular events during the trial (most individuals were young and had a low risk of cardiovascular disease). The results of the DCCT predicted that individuals in the intensive diabetes management group would gain 7.7 additional years of vision, 5.8

additional years free from ESRD, and 5.6 years free from lower extremity amputations. If all complications of DM were combined, individuals in the intensive diabetes management group would experience 15.3 more years of life without significant microvascular or neurologic complications of DM, compared to individuals who received standard therapy. This translates into an additional 5.1 years of life expectancy for individuals in the intensive diabetes management group.[46]

The benefit of the improved glycemic control during the DCCT persisted even after the study concluded and glycemic control worsened. For example, individuals in the intensive diabetes management group for a mean of 6.5 years had a 42–57% reduction in cardiovascular events [nonfatal myocardial infarction (MI), stroke, or death from a cardiovascular event] at a mean follow-up of 17 years, even though their subsequent glycemic control was the same as those in the conventional diabetes management group.[46]

The benefits of an improvement in glycemic control occurred over the entire range of A1C values, suggesting that at any A1C level, an improvement in glycemic control is beneficial. The goal of therapy is to achieve an A1C level as close to normal as possible, without subjecting the patient to excessive risk of hypoglycemia.[46]

The United Kingdom Prospective Diabetes Study (UKPDS) demonstrated that each percentage point reduction in A1C was associated with a 35% reduction in microvascular complications.

Similar reductions in the risks of retinopathy and nephropathy were also seen in a small trial of lean Japanese individuals with type 2 DM randomized to either intensive glycemic control or standard therapy with insulin (Kumamoto study). These results demonstrate the effectiveness of improved glycemic control in individuals of different ethnicity and presumably, a different etiology of DM (that is phenotypically different from those in the UKPDS).[47]

The findings of the UKPDS, and Kumamoto study support the idea that chronic hyperglycemia plays a causative role in the pathogenesis of diabetic microvascular complications. These landmark studies prove the value of metabolic control and emphasize the importance of intensive glycemic control in all forms of DM.[47,48]

DIABETIC RETINOPATHY

Diabetic retinopathy may be the most common microvascular complication of diabetes. It is responsible for ~ 10,000 new cases of blindness every year in the United States alone. The risk of developing diabetic retinopathy or other microvascular complications of diabetes depends on both the duration and the severity of hyperglycemia.[32] Development of diabetic retinopathy in patients with type 2 diabetes was found to be related to both severity of hyperglycemia and presence of hypertension in the U.K. Prospective Diabetes Study (UKPDS).[41,49] Retinopathy may begin to develop as early as 7 years before the diagnosis of diabetes in patients with type 2 diabetes. There are several proposed pathological mechanisms by which diabetes may lead to development of retinopathy.

Aldose reductase may participate in the development of diabetes complications. Aldose reductase is the initial enzyme in the intracellular polyol pathway. This pathway involves the conversion of glucose into glucose alcohol (sorbitol). High glucose levels increase the flux of sugar molecules through the polyol pathway, which causes sorbitol accumulation in cells. Osmotic stress from sorbitol accumulation has been postulated as an underlying mechanism in the development of diabetic microvascular complications, including diabetic retinopathy. In animal models, sugar alcohol accumulation has been linked to microaneurysm formation, thickening of basement membranes, and loss of pericytes. Treatment studies with aldose reductase inhibitors, however, have been disappointing.[32,48]

Cells are also thought to be injured by glycoproteins. High glucose concentrations can promote the nonenzymatic formation of advanced glycosylated end products (AGEs). In animal models, these substances have also been associated with formation of microaneurysms and pericyte loss. Evaluations of AGE inhibitors are underway.[48]

Oxidative stress may also play an important role in cellular injury from hyperglycemia. High glucose levels can stimulate free radical production and reactive oxygen species formation. Animal studies have suggested that treatment with antioxidants, such as vitamin E, may attenuate some vascular dysfunction associated with diabetes, but treatment with antioxidants has not yet been shown to alter the development or progression of retinopathy or other microvascular complications of diabetes.[32,48]

Growth factors, including vascular endothelial growth factor (VEGF), growth hormone, and transforming growth factor β, have also been postulated to play important roles in the development of diabetic retinopathy. VEGF production is increased in diabetic retinopathy, possibly in response to hypoxia. In animal models, suppressing VEGF production is associated with less progression of retinopathy.[32,48]

Diabetic retinopathy is generally classified as either non proliferative or proliferative. It is important to have a general understanding of the features of each to interpret eye examination reports and advise patients of disease progression and prognosis.

CLASSIFICATION (MODIFIED FROM AMERICAN ACADEMY OF OPTHALMOLOGY)[50]

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 on 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,[51]

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.[11]

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/dL 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.

Diabetic nephropathy is the leading cause of renal failure in the United States. It is defined by proteinuria > 500 mg in 24 hours in the setting of diabetes, but this is preceded by lower degrees of proteinuria, or “microalbuminuria.” Microalbuminuria

is defined as albumin excretion of 30-299 mg/24 hours. Without intervention, diabetic patients with microalbuminuria typically progress to proteinuria and overt diabetic nephropathy. This progression occurs in both type 1 and type 2 diabetes.

As many as 7% of patients with type 2 diabetes may already have microalbuminuria at the time they are diagnosed with diabetes.[52] In the European Diabetes Prospective Complications Study, the cumulative incidence of microalbuminuria in patients with type 1 diabetes was ~ 12% during a period of 7 years.[52,53] In the UKPDS, the incidence of microalbuminuria was 2% per year in patients with type 2 diabetes, and the 10-year prevalence after diagnosis was 25%.[47,52]

The pathological changes to the kidney include increased glomerular basement membrane thickness, microaneurysm formation, mesangial nodule formation (Kimmelsteil-Wilson bodies), and other changes. The underlying mechanism of injury may also involve some or all of the same mechanisms as diabetic retinopathy.[32]

Screening for diabetic nephropathy or microalbuminuria may be accomplished by either a 24-hour urine collection or a spot urine measurement of microalbumin. Measurement of the microalbumin-to-creatinine ratio may help account for concentration or dilution of urine, and spot measurements are more convenient for patients than 24-hour urine collections. It is important to note that falsely elevated urine protein levels may be produced by conditions such as urinary tract infections, exercise, and hematuria.[32]

Initial treatment of diabetic nephropathy, as of other complications of diabetes, is prevention. Like other microvascular complications of diabetes, there are strong associations between glucose control (as measured by hemoglobin A_{1c} [A1C]) and the risk of developing diabetic nephropathy. Patients should be treated to the lowest safe glucose level that can be obtained to prevent or control diabetic nephropathy. Treatment with angiotensin-converting enzyme (ACE) inhibitors has not been shown to prevent the development of microalbuminuria in patients with type 1 diabetes but has been shown to decrease the risk of developing nephropathy and cardiovascular events in patients with type 2 diabetes.[32,52,54]

In addition to aggressive treatment of elevated blood glucose, patients with diabetic nephropathy benefit from treatment with antihypertensive drugs. Renin-angiotensin system blockade has additional benefits beyond the simple blood pressure-lowering effect in patients with diabetic nephropathy. Several studies have demonstrated renoprotective effects of treatment with ACE inhibitors and antiotensin receptor blockers (ARBs), which appear to be present independent of their blood pressure-lowering effects, possibly because of decreasing intraglomerular pressure. Both ACE inhibitors and ARBs have been shown to decrease the risk of progression to macroalbuminuria in patients with microalbuminuria by as much as 60-70%. These drugs are recommended as the first-line pharmacological treatment of microalbuminuria, even in patients without hypertension.[52]

Similarly, patients with macroalbuminuria benefit from control of hypertension. Hypertension control in patients with macroalbuminuria from diabetic kidney disease slows decline in glomerular filtration rate (GFR). Treatment with

ACE inhibitors or ARBs has been shown to further decrease the risk of progression of kidney disease, also independent of the blood pressure-lowering effect.

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.[1]

Classification[1]

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)[55]

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.

Diabetic neuropathy is recognized by the American Diabetes Association (ADA) as "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes."[56] As with other microvascular complications, risk of developing diabetic neuropathy is proportional to both the magnitude and duration of hyperglycemia, and some individuals may possess genetic attributes that affect their predisposition to developing such complications.

The precise nature of injury to the peripheral nerves from hyperglycemia is not known but likely is related to mechanisms such as polyol accumulation, injury from AGEs, and oxidative stress.[32]

Chronic sensorimotor distal symmetric polyneuropathy is the most common form of neuropathy in diabetes. Typically, patients experience burning, tingling, and

"electrical" pain, but sometimes they may experience simple numbness. In patients who experience pain, it may be worse at night. Patients with simple numbness can present with a painless foot ulceration, so it is important to realize that lack of symptoms does not rule out presence of neuropathy. Physical examination reveals sensory loss to light touch, vibration, and temperature. Abnormalities in more than one test of peripheral sensation are > 87% sensitive in detecting the presence of neuropathy. Patients also typically experience loss of ankle reflex.[57] Patients who have lost 10-g monofilament sensation are at considerably elevated risk for developing foot ulceration.[58]

Pure sensory neuropathy is relatively rare and associated with periods of poor glycemic control or considerable fluctuation in diabetes control. It is characterized by isolated sensory findings without signs of motor neuropathy. Symptoms are typically most prominent at night.[57]

Mononeuropathies typically have a more sudden onset and involve virtually any nerve, but most commonly the median, ulnar, and radial nerves are affected. Cranial neuropathies have been described but are rare. Diabetic amyotrophy may be a manifestation of diabetic mononeuropathy and is characterized by severe pain and muscle weakness and atrophy, usually in large thigh muscles.[57]

Several other forms of neuropathy that mimic the findings in diabetic sensory neuropathy and mononeuropathy like Chronic inflammatory polyneuropathy, vitamin B_{12} deficiency, hypothyroidism, and uremia should be ruled out in the process of evaluating diabetic peripheral neuropathy.[57]

Diabetic autonomic neuropathy also causes significant morbidity and even mortality in patients with diabetes. Neurological dysfunction may occur in most organ systems and can by manifest by gastroparesis, constipation, diarrhea, anhidrosis, bladder dysfunction, erectile dysfunction, exercise intolerance, resting tachycardia, silent ischemia, and even sudden cardiac death.[57] Cardiovascular autonomic dysfunction is associated with increased risk of silent myocardial ischemia and mortality.[59]

There is no specific treatment of diabetic neuropathy, although many drugs are available to treat its symptoms. The primary goal of therapy is to control symptoms and prevent worsening of neuropathy through improved glycemic control. Some studies have suggested that control of hyperglycemia and avoidance of glycemic excursions may improve symptoms of peripheral neuropathy. Amitriptyline, imiprimine, paroxetine, citalopram, gabapentin, pregablin, carbamazepine, topiramate, duloxetine, tramadol, and oxycodone have all been used to treat painful symptoms, but only duloxetine and pregablin possess official indications for the treatment of painful peripheral diabetic neuropathy.[57]

Treatment with some of these medications may be limited by side effects of the medication, and no single drug is universally effective. Treatment of autonomic neuropathy is targeted toward the organ system that is affected, but also includes optimization of glycemic control.[32]

Magnesium

Magnesium is the fourth most abundant cation in the body and second most abundant intracellularly, after potassium. Adult human body contains 21-28gms

(approximately 2000mEq) of magnesium. Approximately 60% of total body magnesium is located in bone and the remainder is in soft tissues. This soft tissue intracellular compartment comprises about 38% of total body magnesium; relatively higher concentrations are found in liver and skeletal muscle. (15-20 mEq/Kg). Less than 2% is present in extracellular fluid (ECF) compartment.[60]

Serum concentration of magnesium ranges from 1.8 to 2.5 mg/dL. (0.7 to 1.0 mmol/L). The plasma concentration in healthy adults remain remarkably constant.[7] It is important to point out that limits of the normal range deviate from the mean by less than 15%, indicating that the serum concentration is maintained by sensitive control mechanisms that are poorly understood at present.

The average daily intake of magnesium is of the order of 25 mEq (140-360 mg/day). Less than 40% of dietary magnesium is absorbed throughout the small intestine predominantly in the ileum. Elimination is predominantly renal and averages 100mg/day. The threshold for urinary excretion is near the upper limit of normal range. Thus when serum levels rise above 2.5 mg/dL, magnesium excretion increases dramatically. Conversely, the kidney retains a strong capacity to reabsorb magnesium in condition of magnesium depletion, and the main site for reabsorption is the thick ascending loop of Henle. Several factors may impair renal reabsorption, such as volume expansion, hypercalcemia, and diuretic administration (eg. osmotic, thiazide or loop).[1]

Biochemical importance of magnesium

Magnesium is an activator of a host of enzyme systems that are critical to cellular metabolism. Prominent are the enzymes that hydrolyze and transfer

phosphate groups, especially those involved in the reactions involving adenosine triphosphate (ATP). As ATP is required for glucose utilization, fat, protein, nucleic acid and co-enzyme synthesis, muscle contraction and other reactions, by inference the activating effect of magnesium extends to all these functions.[61]

Additionally, magnesium is required as a cofactor for oxidative phosphorylation in the mitochondria.[62]

Magnesium contributes importantly to macromolecular structure. The highly ordered organization of DNA, RNA and ribosomes is stabilised by presence of this metal.[63,64] Magnesium is further involved in protein synthesis by contributing to the binding of messenger RNA to the 70s ribosome.[65]

Interrelations of Major Biologic Cations

- Magnesium is generally found in high concentrations within the cell, where as intracellular calcium content is low. The ratios are inverted in extracellular fluid.[66]
- Variations in dietary calcium does not affect the absorption of magnesium.
- Magnesium reabsorption in renal tubules is inhibited by hypercalcemia.
- The intracellular phosphate concentration is usually parallel to magnesium concentration.

Regulation of Serum Magnesium

Renal regulation

Regulation of serum magnesium concentration is mainly achieved by control of renal magnesium reabsorption. Only 20% of the filtered magnesium is reabsorbed

in the proximal tubule, where as 60% is reclaimed in the cTAL (thick ascending loop) and another 5-10% in DCT (Distal tubule). Magnesium reabsorption in cTAL is increased by parathyroid hormone and is inhibited by hypercalcemia and hypermagnesemia.[67]

Intestinal absorption

Around 30-40% of dietary magnesium (normally ranges from 140 – 360 mg/day) is absorbed, mainly in the jejunum and ileum. Intestinal magnesium absorptive efficiency is stimulated by 1,25 $(OH)_2$ Vitamin D and can reach 70% during magnesium deprivation.[67,68]

Hormonal factors

Increasing serum magnesium,[68]

- Parathyroid hormone
- Glucagon
- 1, 25$(OH)_2$ Calcitriol.

Decreasing serum magnesium,

- Aldosterone
- Vasopressin (ADH)
- Thyroxine
- Calcitonin

Normal Mg Metabolism

Gastrointestinal Metabolism

On an average American diet, 250 to 350 mg of Mg is consumed daily. Twenty-five to 60% of dietary Mg is absorbed in the gastrointestinal tract. Gastrointestinal absorption occurs predominantly in the small intestines *via* paracellular simple diffusion at high intraluminal concentrations and active transcellular uptake *via* Mg-specific transporters at low concentrations.[69] Active intestinal Mg absorption is presumed to involve transient receptor potential channel melastatin 6 (TRPM6), which is expressed along the brush border membrane of the small intestine.[70] Mutations of TRPM6 have been reported to be associated with hypomagnesemia with secondary hypocalcemia.[13]

Renal Metabolism

Glomerular Filtration

Approximately 70 to 80% of plasma Mg is ultrafilterable in the ionic form (70 to 80%) and complexed with anions such as phosphate, citrate, and oxalate (20 to 30%). The ultrafilterability of Mg depends on glomerular filtration, volume status, various metabolic states that would enhance the selection for ionized Mg (*e.g.*, acidemia, reduced serum content of negatively charged species), and the integrity of the glomerular basement membrane.[70]

Proximal Tubules

Once Mg is filtered through the glomerulus, 15 to 25% is reabsorbed in the proximal tubules. Reabsorption at the proximal tubule is mainly passive and proportional to sodium and water reabsorption, although at a lower rate.[70]

Loop of Henle.

Approximately 65 to 75% of the Mg filtered load is reabsorbed *via* the paracellular pathway in the thick ascending limb of the loop of Henle (TAL). Paracellular Mg reabsorption at this nephron segment has been suggested to be facilitated by claudin 6, also known as paracellin 1. Paracellin 1 is a tight junction protein whose mutation is associated with severe hypomagnesemia with hypercalciuria and nephrolithiasis. Parathyroid hormone, calcitonin, glucagon, and antidiuretic hormone have been suggested to enhance Mg transport in the TAL *via* the second messenger cAMP. Insulin also has been implicated to play a role at this nephron segment by increasing the favorable transepithelial potential difference for Mg reabsorption.[70]

Distal Convoluted Tubules

The distal convoluted tubule (DCT) reabsorbs approximately 5 to 10% of the filtered Mg *via* an active and regulated transcellular pathway. Although this is a low percentage of the filtered Mg load, it represents 70 to 80% of Mg that is delivered from the TAL. In addition, because a negligible amount of Mg is reabsorbed distal to this segment, Mg reabsorption at the DCT is of great importance because it determines the final urinary Mg concentration.[70]

Recently, Mg reabsorption at the DCT was shown to occur *via* the transient receptor potential channel melastatin TRPM6. It has been postulated that upon entry into the cells, Mg binds to divalent-binding proteins such as parvalbumin or calbindin-D28K for transport across the cell to the basolateral membrane, where Mg is taken into the interstitium by a basolateral Na2_/Mg2_ exchanger and/or ATPdependent Mg pump.[70]

It is interesting that the regulation of magnesium reabsorption at the DCT was studied extensively before the actual identification of TRPM6. Peptide hormones such as parathyroid hormone (PTH), calcitonin, glucagon, and vasopressin all have been implicated. The mediating mechanisms are unknown but seem to involve, in part, stimulation of cAMP release and activation of protein kinase A, phospholipase C, and protein kinase C. Insulin also has been suggested to enhance intracellular Mg uptake, presumably *via* tyrosine kinase. Moreover, insulin may stimulate the production of cAMP and potentiate Mg uptake *via* other cAMP-dependent hormones, including PTH (62). In addition, the Ca2_/Mg2_ sensing receptor on the basolateral side may modulate hormone-stimulated Mg transport through G-protein coupling. Finally, low dietary Mg intake and estrogens have been shown to upregulate renal TRPM6 expression and reduce urinary Mg excretion.[70]

Dietary Reference Intakes for Magnesium

Recommendations for magnesium are provided in the dietary reference intakes (DRI's) developed by the Food and Drug Administration (FDA).[71,72]

Hypomagnesemia

Hypomagnesemia signifies substantial depletion of body magnesium stores (0.5 to 1 mmol/Kg). Hypomagnesemia has varied etiology. Dietary magnesium deficiency is unlikely except in the setting of alcoholism.[1]

Causes of Hypomagensemia[1]

I. Impaired intestinal absorption
   A. Primary infantile hypomagnesemia
   B. Malabsorption syndromes
   C. Vitamin D deficiency.

II. Increased intestinal losses
   A. Protracted vomiting / diarrhea
   B. Intestinal drainage, fistulae

III. Impaired renal tubular reabsorption
   A. Genetic magnesium wasting syndromes.
      1. Gitelman syndrome
      2. Bartter syndrome
      3. Na-K ATPase g-subunit mutations
   B. Acquired renal disease
      1. Tubulointerstitial disease
      2. Post obstruction /ATN (diuretic phase)
      3. Renal transplantation.
   C. DRUGS
      1. Ethanol
      2. Diuretics (loop, thiazide and osmotic)

3. Cisplatin, cyclosporine
4. Aminoglycosides, Amphotericin B

IV. Metabolic causes

1. Hyperaldosteronism
2. SIADH
3. Diabetes mellitus
4. Metabolic acidosis
5. Hypercalcemia
6. Hyperthyroidism

V. OTHERS

1. Pancreatitis
2. Excessive sweating
3. Osteoblastic metastases

Several genetic magnesium wasting syndromes are explained, but are extremely rare. Prolonged nasogastric suction, parenteral fluids, infectious diarrhea, steatorrhoea, inflammatory bowel disease may cause hypomagnesemia.[33] Magnesium deficiency is especially common in patients receiving furosemide diuretic.[73]

Magnesium and diabetes

Magnesium ion has a fundamental role in carbohydrate metabolism and Diabetes mellitus has been suggested to be the most common metabolic disorder associated with magnesium deficiency, which has 25 to 39% prevalence in diabetes.[62,67,74,75]

Hypomagnesemia, defined by low serum Mg concentrations, has been reported to occur in 13.5 to 47.7% of nonhospitalized patients with type 2 diabetes compared with 2.5 to 15% among their counterparts without diabetes.[13,76] The wide range in the reported incidence of hypomagnesemia most likely reflects the difference in the definition of hypomagnesemia, techniques in Mg measurements, and the heterogeneity of the selected patient cohort. In terms of gender difference, it is interesting to note that independent studies have reported a higher incidence of hypomagnesemia in women compared with men, at a 2-to-1 ratio. In addition, men with diabetes may have higher ionized levels of Mg.[70]

Hypomagnesemia and Diabetes: Cause and Effect

Not only has hypomagnesemia been associated with type 2 diabetes, but also numerous studies have reported an inverse relationship between glycemic control and serum Mg levels. Although many authors have suggested that diabetes *per se* may induce hypomagnesemia, others have reported that higher Mg intake may confer a lower risk for type 2 diabetes. It is interesting that the induction of Mg deficiency has been shown to reduce insulin sensitivity in individuals without diabetes, whereas Mg supplementation during a 4-wk period has been shown to improve glucose handling in elderly individuals without diabetes. In patients with type 2 diabetes, oral Mg supplementation during a 16-wk period was suggested to improve insulin sensitivity and metabolic control. The mechanisms whereby hypomagnesemia may induce or worsen existing diabetes are not well understood.[70]

Nonetheless, it has been suggested that hypomagnesemia may induce altered cellular glucose transport, reduced pancreatic insulin secretion, defective

postreceptor insulin signaling, and/or altered insulin–insulin receptor interactions. Not all studies, however, observed a correlation between glycemic control and serum Mg levels or improvement of diabetic control with Mg replacement.[70]

Hypomagnesemia and Adverse Clinical Associations in Type 2 Diabetes

Hypomagnesemia at the Cellular Level

There is considerable evidence to suggest that hypomagnesemia may adversely affect various aspects of cellular physiology. Available data suggest that low Mg levels may promote endothelial cell dysfunction and thrombogenesis *via* increased platelet aggregation and vascular calcifications.[77] Low Mg levels also may lead to the induction of proinflammatory and profibrogenic response, reduction of protective enzymes against oxidative stress, induction or augmentation of vasoconstriction and hypertension, and stimulation of aldosterone, among others. Moreover, because Mg is crucial in DNA synthesis and repair, it is possible that Mg deficiency may interfere with normal cell growth and regulation of apoptosis.[70]

Hypomagnesemia in the Clinical Setting

Clinically, there are significant data linking hypomagnesemia to various diabetic micro- and macrovascular complications.[70]

Cardiovascular

In a study that involved 19 normotensive individuals without diabetes, 17 hypertensive individuals without diabetes, and 6 hypertensive individuals with diabetes, Resnick et al.[78] documented the lowest mean intracellular Mg concentration among the last group.

Similarly, based on data from the Atherosclerosis Risk in Communities (ARIC) Study, a multicenter, prospective cohort study that lasted 4 to 7 yr and involved 13,922 middle-aged adults who were free of coronary heart disease at baseline, an inverse association between serum Mg and the risk for coronary heart disease was observed among men with diabetes.[79]

Diabetic Retinopathy

The link between hypomagnesemia and diabetic retinopathy was reported in two cross-sectional studies that involved both "insulin-dependent" patients and patients with type 2 diabetes. Not only did patients with diabetes have lower serum Mg levels compared with their counterparts without diabetes, but also the serum Mg levels among the cohort with diabetes had an inverse correlation with the degree of retinopathy.[80,81] A similar link, however, was not observed when Mg was measured within mononuclear cells. In a study that involved 128 patients with type 2 diabetes and poor glycemic control (glycosylated hemoglobin >8.0%), intramononuclear Mg concentrations were not observed to be lower among those with diabetic retinopathy but rather among those with neuropathy and coronary disease.[82]

Foot Ulcerations.

Given the link between hypomagnesemia and risk factors for the development of diabetic foot ulcers (*e.g.*, polyneuropathy, platelet dysfunction), Rodriguez- Moran and Guerrero-Romero[83] suggested that hypomagnesemia may be associated with an increased risk of diabetic foot ulcers. Indeed, they observed a higher incidence of hypomagnesemia among their patients with diabetic foot ulcers compared with those without the condition (93.9% of the 33 patients with diabetic

foot ulcers compared with 73.1% of the 66 patients without diabetic foot ulcers; p=0.02).

Nephropathy

In a comparative study that involved 30 patients who had type 2 diabetes without microalbuminuria, 30 with microalbuminuria, and 30 with overt proteinuria, Corsonello et al.[84] observed a significant decrease in serum ionized Mg in both the microalbuminuria and overt proteinuria groups compared with the nonmicroalbuminuric group. Accordingly, in a recent retrospective study, an association between low serum Mg levels and a significantly faster rate of renal function deterioration in patients with type 2 diabetes was reported.[70]

Others

Finally, there also are data to suggest the association between hypomagnesemia and other diabetic complications, including dyslipidemia and neurologic abnormalities. Because hypomagnesemia has been linked to various microand macrovascular complications, a better understanding of Mg metabolism and efforts to minimize hypomagnesemia in the routine management of diabetes are warranted.[70]

Possible Causes of Hypomagnesemia in Type 2 Diabetes

Hypomagnesemia in the patient with diabetes may result from poor oral intake, poor gastrointestinal absorption, and enhanced renal Mg excretion.

Possible causes of hypomagnesemia in patients with type 2 diabetes[70]

- Decreased intake
    - Poor oral intake
    - Esophageal dysfunction
    - Diabetic gastroparesis
- Enhanced gastrointestinal loss diarrhea as a result of autonomic dysfunction
- Enhanced renal magnesium loss
    - Enhanced filtered load
        - Glomerular hyperfiltration
        - Osmotic diuresis (glucosuria)
        - Volume expansion as a result of excessive volume replacement
        - Metabolic acidosis (diabetic ketoacidosis)
        - Hypoalbuminemia
        - Microalbuminuria and overt proteinuria
    - Reduced renal reabsorption
        - Endocrinologic dysfunction: insulin deficiency or resistance
        - Metabolic acidosis (diabetic ketoacidosis)
        - Electrolyte abnormalities: phosphate and potassium depletion
        - Diuretics
        - Others

Gastrointestinal Causes

Diabetic autonomic neuropathies that may reduce oral intake and gastrointestinal absorption include esophageal dysfunction, gastroparesis, and

diarrhea.[57,85] Whether gastrointestinal Mg absorption *via* TRPM6 is reduced in the patient with diabetes is not known.

Renal Causes

Enhanced Filtered Load

In the patient with diabetes, the ultrafilterable Mg load may be enhanced by glomerular hyperfiltration, recurrent excessive volume repletion after hyperglycemia- induced osmotic diuresis, recurrent metabolic acidosis associated with diabetic ketoacidosis, and hypoalbuminemia.[69] The last two conditions may increase the serum ionized Mg fraction and, hence, ultrafilterable Mg load and subsequent urinary loss. In addition, it is conceivable that significant microalbuminuria and overt proteinuria among patients with diabetic nephropathy may contribute to renal Mg wasting as a result of protein-bound magnesium loss.[70]

Enhanced Tubular Flow

Overly aggressive volume reexpansion and glomerular hyperfiltration also may induce renal Mg wasting at the proximal tubule and TAL, independent of the filtered load. Because Mg reabsorption parallels sodium reabsorption in the proximal tubules, volume expansion can decrease both sodium and Mg reabsorption at this level. Similarly, a high tubular flow through the TAL may reduce Mg reabsorption at this segment.[69]

Reduced Tubular Reabsorption.

Because insulin has been implicated in enhancing Mg reabsorption at the TAL, insulin deficiency or resistance in the diabetic state can promote Mg wasting

at this nephron segment.[86] The expression of paracellin 1 in TAL, however, has not been shown to be increased in diabetic rats.[70]

In the same diabetic rat model, Lee et al.[87] revealed that TRPM6 expression in the DCT is not reduced but rather enhanced. This is thought to be a compensatory mechanism for the increased Mg load that is delivered to the DCT or blunted activity of the TRPM6 channel in the diabetic state. Accordingly, despite the increase in TRPM6 expression, overall renal Mg wasting is observed.

Metabolic Disturbances

Various metabolic disturbances that are associated with diabetes also have been suggested to promote urinary Mg excretion.[70]

Hypokalemia

At the TAL segment, hypokalemia may reduce Na^+-K^+-$2Cl^-$ co-transport activity, the associated potassium extrusion through the potassium channel ROMK, and resultant diminution of the favorable transmembrane voltage that is required for paracellular Mg reabsorption. In addition, there is evidence to suggest that cellular potassium depletion may diminish Mg reabsorption at the DCT by yet unclear mechanisms.[88]

Hypophosphatemia.

Both micropuncture studies in phosphate- depleted dogs and *in vitro* studies involving phosphatedepleted mouse DCT cells have demonstrated reduced Mg uptake. Phosphate-induced reduction in cellular uptake of Mg is believed to be a

posttranslational effect because the alteration in Mg uptake could be observed within 30 min of phosphate depletion.[70]

Metabolic Acidosis

In addition to its role in increasing serum ionized Mg concentration and, hence, ultrafilterable Mg load for renal excretion, metabolic acidosis has been suggested to enhance protonation of the Mg channel in the DCT and subsequent inhibition of cellular Mg uptake.[70] More recently, Nijenhuis et al.[89] showed reduced expression of TRPM6 with induced chronic metabolic acidosis in mice.

Insulin Deficiency and/or Resistance

As previously discussed, insulin deficiency or resistance may exacerbate renal Mg wasting because insulin has been shown to have antimagnesiuric effects in both the TAL and the DCT.[70]

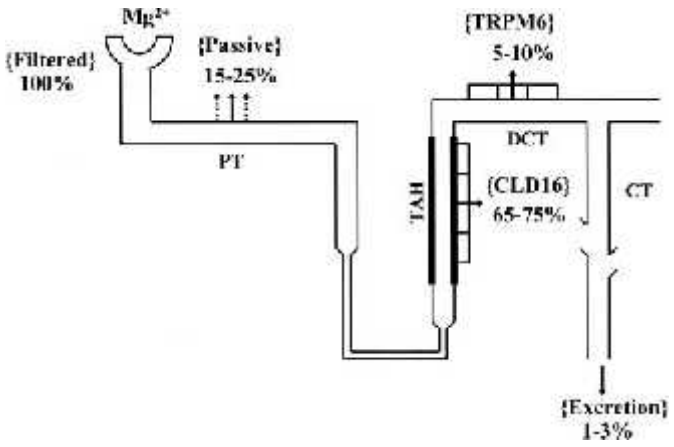


Figure 4. Renal magnesium (Mg) handling. After glomerular filtration, ionized magnesium is reabsorbed passively in parallel to sodium reabsorption at the proximal tubules (PT); paracellularly *via* claudin 6 (CLD16; paracellin 1) at the thick ascending limb of the loop of Henle (TAH); and transcellularly *via* transient receptor potential channel melastatin (TRPM6) at the distal convoluted tubule (DCT). CT, collecting tubules.[70]

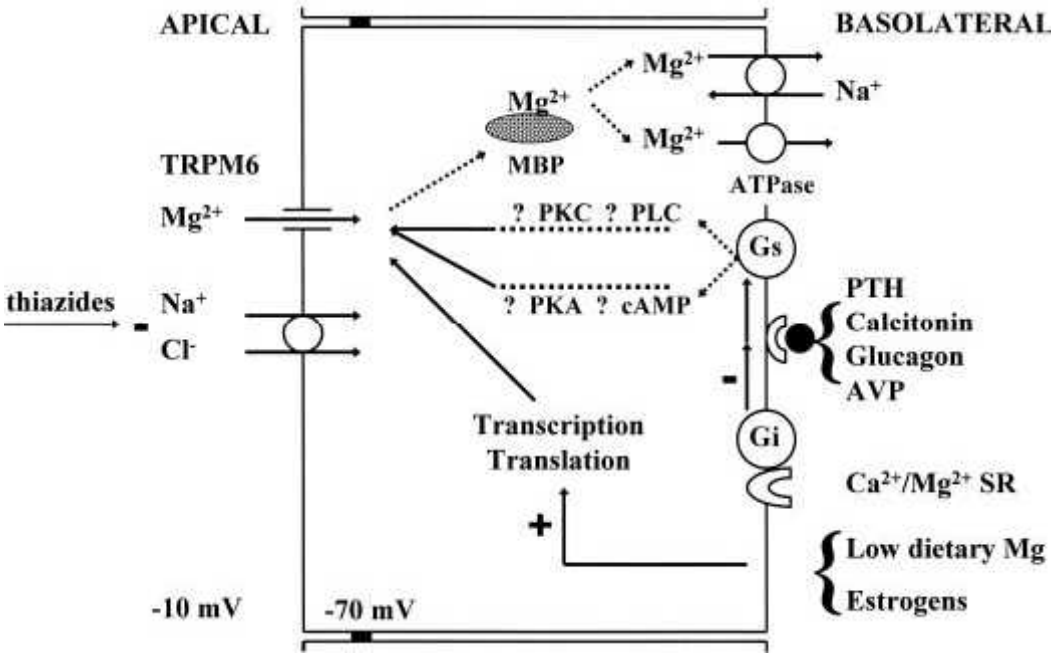


Figure 5. Regulation of Mg handling at the DCT. AVP, arginine vasopressin; Ca2_/Mg2_ SR, Ca2_/Mg2_ sensing receptor; Gi, inhibitory G protein; Gs, stimulatory G protein; MPB, Mg2_- binding protein; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PTH, parathyroid hormone. Adapted from reference (62), with permission[70]

Use of Diuretics

The common use of diuretics among patients with diabetes also may contribute to magnesiuria. The degree of magnesiuria is traditionally thought to be lower for thiazides compared with loop diuretics. This difference has been explained by the site of action of the two types of diuretics because a smaller amount of intraluminal Mg is available for wasting at the DCT compared with that at the loop of Henle. In addition, inhibition of the Na^+-Cl^- co-transporter by thiazides has been suggested to induce hyperpolarization of the DCT plasma membrane and, hence, a more favorable transmembrane electrical gradient for Mg reabsorption.[70]

Despite these theoretical advantages of thiazides over loop diuretics, severe hypomagnesemia is observed more frequently with Gitelman's compared with Bartter's syndrome, two syndromes that have traditionally been equated to the administration of thiazides and furosemide, respectively. Recently, in support of this observation, reduced TRPM6 expression and enhanced magnesiuria were shown in mice given chronic thiazide therapy.[90]

Given these observations and the lack of good direct comparative data between the two classes of diuretics, it must be assumed that significant magnesiuria may occur with either.[70]

Others

Finally, the more common use of antibiotics and antifungals such as aminoglycosides and amphotericin in patients with diabetes may also contribute to renal Mg wasting.[70]

Gupta AD et al.[12] studied 150 non critically ill type 2 diabetics and found that low serum magnesium level was seen in 11.33% of the patients and was associated with poor glycemic control and increased incidence of retinopathy, nephropathy and foot ulcers. Thus it is prudent to monitor magnesium levels in all type 2 diabetic patients on a regular basis.

Prabodh S. et al.[91] conducted a study to know the status of magnesium(Mg) and copper(Cu) in south India with 40 patients of diabetic nephropathy as cases and 40 age, sex matched healthy individuals as controls.they concluded that the mean magnesium level of cases (1.6 $\pm$ 0.32meq/L) were significantly lower than controls(2.14 $\pm$ 0. 16meq/L) [$p<0.05$].But mean Cu level showed no significant

difference with controls and concluded that hypomagnesemia may be linked with development of diabetic nephropathy

Kundu D et al.[92] studied 120 type 2 diabetics with and without retinopathy and found that hypomagnesemia and albuminuria, individually or in conjunction serve as indicators for dysglycemia and could be used as markers for the development of diabetic retinopathy.

Pham PC et al.[13] in their study suggested that, Hypomagnesemia is common among patients with type 2 diabetes and the contributory mechanisms most likely are multifactorial. Because available data suggest that adverse outcomes are associated with hypomagnesemia, it is prudent that routine surveillance for hypomagnesemia be done and the condition be treated whenever possible.

Sakaguchi Y. et al.[93] studied 144 patients with diabetic nephropathy and found that patients with hypomagnesemis had a 2.12 fold higher rate of ESRD. Hypomagnesemia was found to be a novel predictor of ESRD in patients with type 2 diabetic nephropathy.

METHODOLOGY

The present study was carried out in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on patients with Type 2 DM.

Study design

The study design was a hospital based cross-sectional study.

Study period

The present study was conducted from January 2014 to December 2014.

Source of Data

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

Sample size

A total of 150 patients with type 2 diabetes mellitus were included in the study.

Sampling procedure

The sample size (n) was calculated using the following formula:

$$n = Z\alpha^2 \, p \, q \, / \, d^2$$

Where,

n = Sample size

Zα = Constant (1.96)

p = Prevalence (11.93% as obtained from previous study)[92]

q = 100 – p (88.07%)

d = Absolute error (7%)

Therefore;

$n = 1.96^2 \times 11.93 \times 88.07 / 7$[92]

n = 82.37

Based on this formula minimum sample of 83 patients was planned. However, 150 patients fulfilled the selection criteria and hence were enrolled.

Selection criteria

Inclusion Criteria

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

Exclusion Criteria

The following patients were excluded from the study.

- Non diabetic kidney disease
- Chronic diarrhea
- Thyroid dysfunction

- Sepsis
- Chronic alcoholics
- Pregnancy and lactation

Ethical clearance

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

Informed consent

Patients presenting with type 2 diabetes mellitus were screened for the eligibility. The patients fulfilling the selection criteria were briefed about the nature of study and included in the study after obtaining a written informed consent (Annexure–I).

Data collection

Patients were interviewed to obtain the demographic characteristics such as age and sex, presenting complaints, diabetic history and history of other comorbid conditions. These patients were subjected to clinical examination and the findings including vitals and systemic examination findings were noted. Patients were evaluated for the features of diabetic peripheral neuropathy. These findings were recorded on a predesigned and pretested proforma (Annexure-II).

Investigations

Patients were subjected to following investigations.

- Complete haemogram

- Blood sugar levels
    - Fasting blood sugars
    - Post Prandial blood sugars
- Glycosylated hemoglobin.
- Renal function tests
    - Blood Urea
    - Serum Creatinine
- Thyroid functions test
- Urine analysis
    - Albumin
    - Glucose
- Serum Magnesium

Estimation of serum magnesium[94]

Specific tests for serum Magnesium was done by collecting the blood in yellow capped plastic tube (plain/serum separator tube SST). Magnesium was estimated using the colorimetric method that uses calmagite dye. The reference serum or plasma magnesium level by this method is 1.8 - 2.5 mg/dL. The principle of this method is that magnesium forms a purple coloured complex when treated with Calmagite dye in alkaline solution, chelating agent and detergent present in the reagent will help out interference occurring from Calcium and Proteins. The intensity of the purple colour is proportional to magnesium concentration.

Criteria for the diagnosis of microvascular complications

Diabetic retinopathy

Fundoscopy

Dilated fundus examination was done in all the patients using the indirect ophthalmoscope and patients were classified into:

1. No evidence of diabetic retinopathy
2. Non proliferative diabetic retinopathy (NPDR)
3. Proliferative diabetic retinopathy (PDR)

Microalbuminuria for nephropathy[1]

Microalbuminuria was assessed using the microalbumin test kit (Immunoturbidimetric assay) which is a quantitative assay for microalbuminuria. The principle of this method is that, anti-human Urine microalbumin antibodies when mixed with samples containing Urine microalbumin, form insoluble complexes. These complexes cause an absorbance change, dependent upon the Urine microalbumin concentration of the patient sample that can be quantified.

Diabetic neuropathy[1,95]

Diabetic neuropathy was assessed based on:

a. Symptoms of neuropathy like tingling and numbness, nocturnal pains and sensory ataxia.

b. Evaluating sensory modalities like pin prick, vibration using 128Hz tuning fork. Absent or reduced perception was recorded.

c. Patellar and ankle reflexes were evaluated using a tendon hammer together with muscle strength in quadriceps femoris and tibialis anterior.

d. Monofilament test: Done using 10-g Semmes-Weinstein monofilament. It is an inexpensive, easy-to-use, and portable test for assessing the loss of protective sensation, and it is recommended by several practice guidelines to detect peripheral neuropathy in otherwise normal feet. The filament is placed for 2 seconds on the patient's skin of the sole (9 points) and 1 point on the dorsum of the foot; The points at which the patient is able to detect the stress of the filament is counted. A score of 6 or less is abnormal and taken as an indicator for neuropathy. The 5.07/10-g monofilament has been described as the best indicator to determine loss of protective sensation.[96]

A monofilament test score of 6 or less with or without other manifestations (a-c) was taken as an indicator for neuropathy.[96]

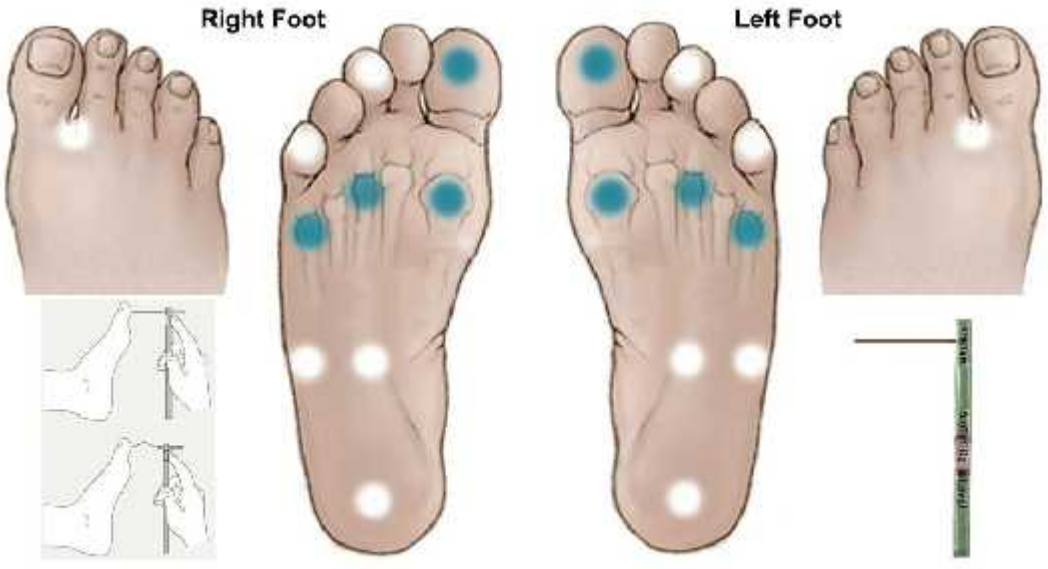


Figure 6. Monofilament test (10 g) for the assessment of diabetic peripheral neuropathy

Statistical methods

The data obtained was coded and entered into the Microsoft Excel Spreadsheet (Annexure III). The data was analysed using SPSS statistical software version 20.0. The categorical data was expressed in terms of rates, ratios and percentages. The comparison of categorical data was done using Chi square or Fisher's exact test and the continuous data was compared using independent student 't' test. More than three means were compared by one way analysis of variance (ANOVA). A 'p' value (probability value) of less than or equal to 0.050 at 95% confidence interval was considered as statistically significant.

RESULTS

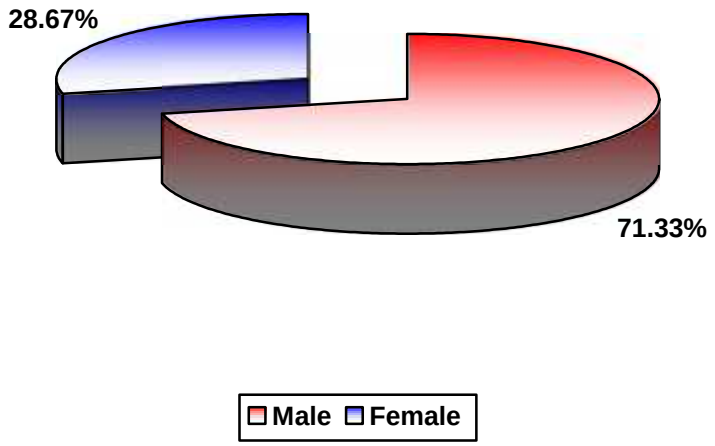
This one year hospital based cross-sectional study was done in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum from January 2014 to December 2014. A total of 150 patients with type 2 diabetes mellitus were studied.

The data obtained was analysed and the final results and observations were tabulated as below.

Table 1. Sex distribution

Sex distribution	Distribution (n=150)	
	Number	**Percentage**
Male	107	71.33
Female	43	28.67
Total	**150**	**100.00**

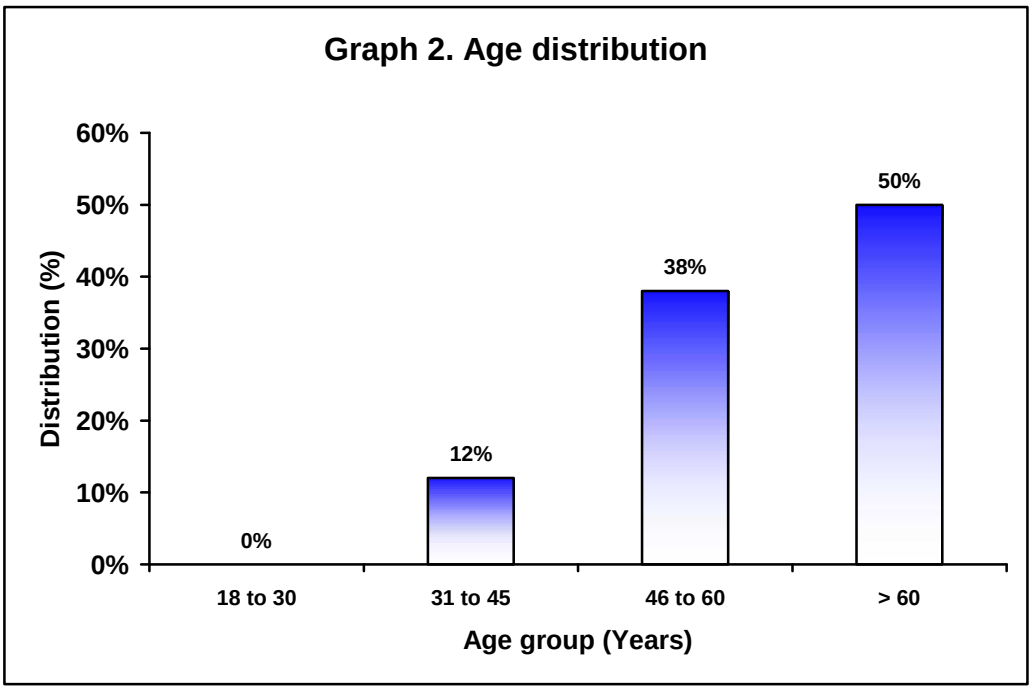
Graph 1. Sex distribution



In the present study 71.33% were males and 28.67% were females. The male to female ration was 2.48:1.

Table 2. Age distribution

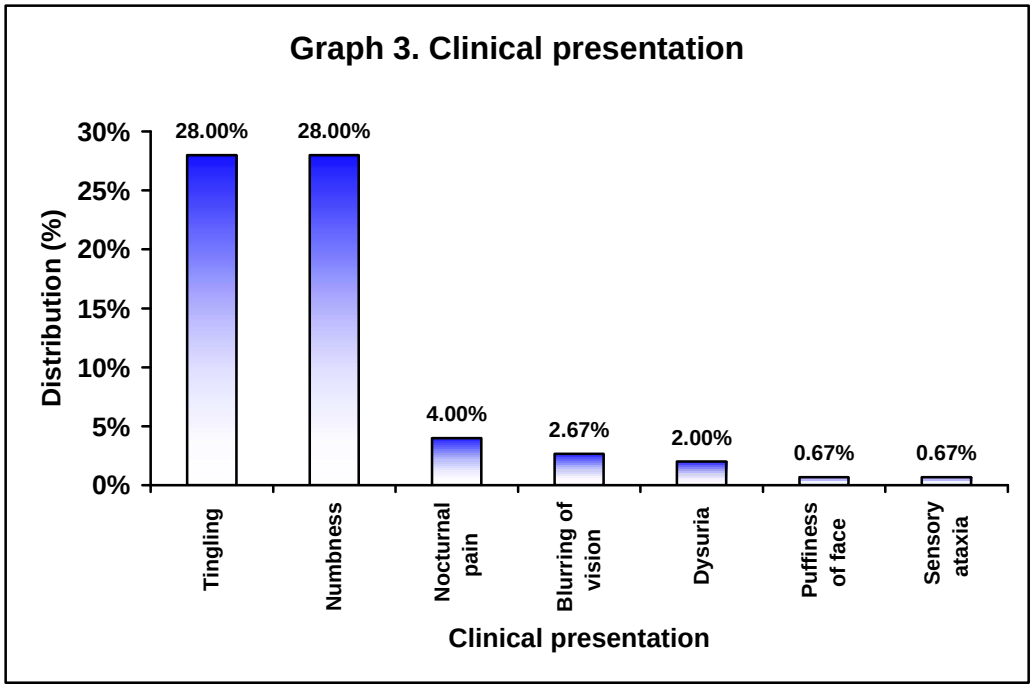
Age group (Years)	Distribution (n=150)	
	Number	**Percentage**
18 to 30	0	0.00
31 to 45	18	12.00
46 to 60	57	38.00
> 60	75	50.00
Total	**150**	**100.00**



In this study the commonest age group for type 2 DM was more than 60 years which comprised of 50% of the patients. Amongst the rest, 38% were aged between 46 to 60 years and 12% between 31 to 45 years. The mean age was 60.38 ± 10.81 years and median age was 60.5 years (Range 36-89 years).

Table 3. Clinical presentation

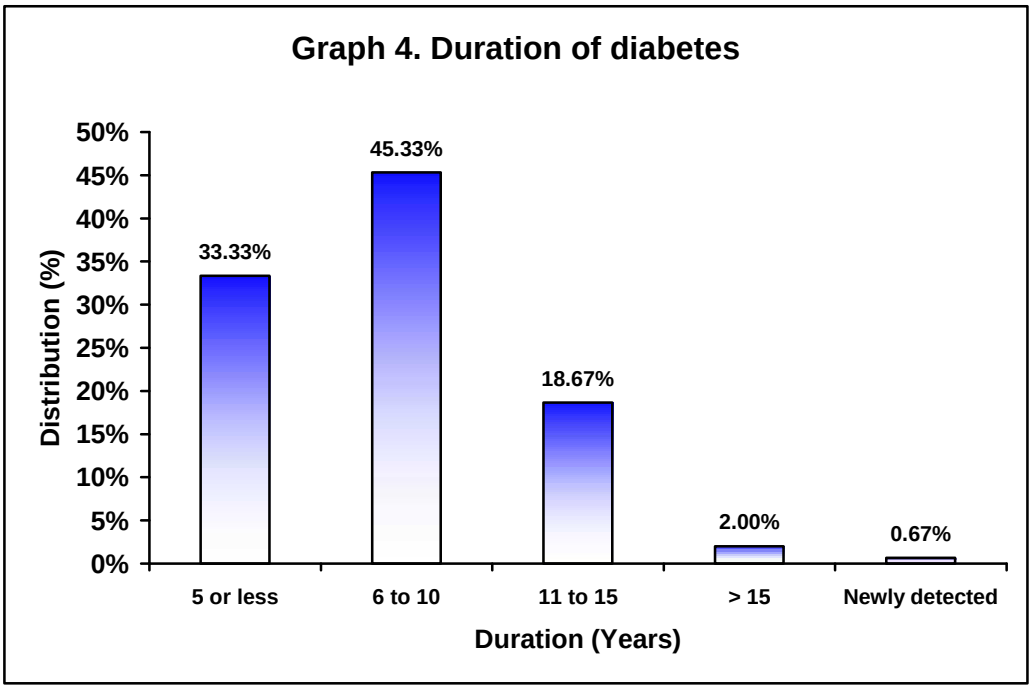
Clinical presentation	Distribution (n=150)	
	Number	Percentage
Tingling	42	28.00
Numbness	42	28.00
Nocturnal pain	6	4.00
Blurring of vision	4	2.67
Dysuria	3	2.00
Puffiness of face	1	0.67
Sensory ataxia	1	0.67



In the present study most common presentation of complications was tingling and numbness i.e symptoms suggestive of neuropathy (28%).

Table 4. Duration of diabetes

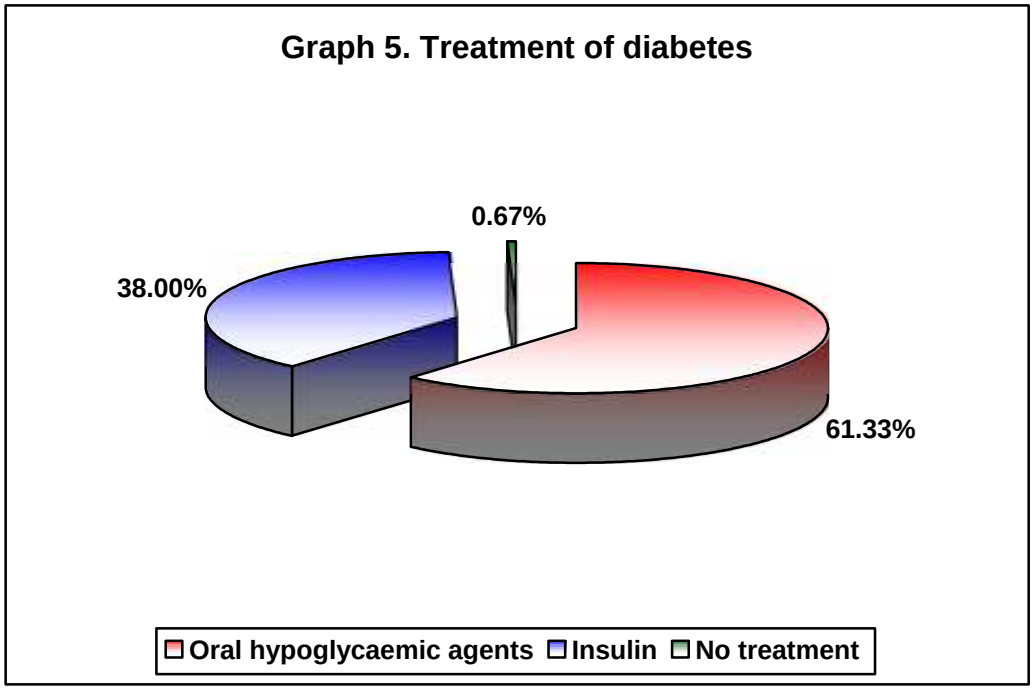
Duration (Years)	Distribution (n=150)	
	Number	**Percentage**
5 or less	50	33.33
6 to 10	68	45.33
11 to 15	28	18.67
> 15	3	2.00
Newly detected	1	0.67
Total	**150**	**100.00**



In the present study, 45.33% of the patients had a duration of diabetes between 6 to 10 years. The mean duration was 7.43 ± 4.11 years and the median duration was 7 years with range being 6 months to 22 years.

Table 5. Treatment of diabetes

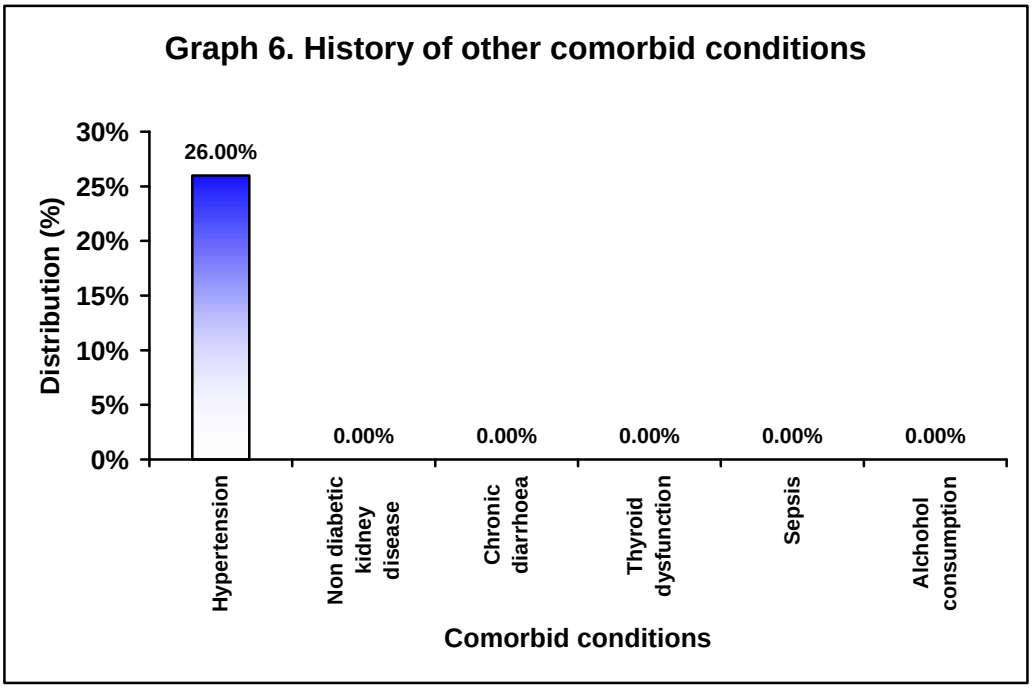
Treatment	Distribution (n=150)	
	Number	**Percentage**
Oral hypoglycaemic agents	92	61.33
Insulin	57	38.00
No treatment	1	0.67
Total	**150**	**100.00**



In the present study most of the patients were on oral hypoglycaemic agents (61.33%).

Table 6. History of other comorbid conditions

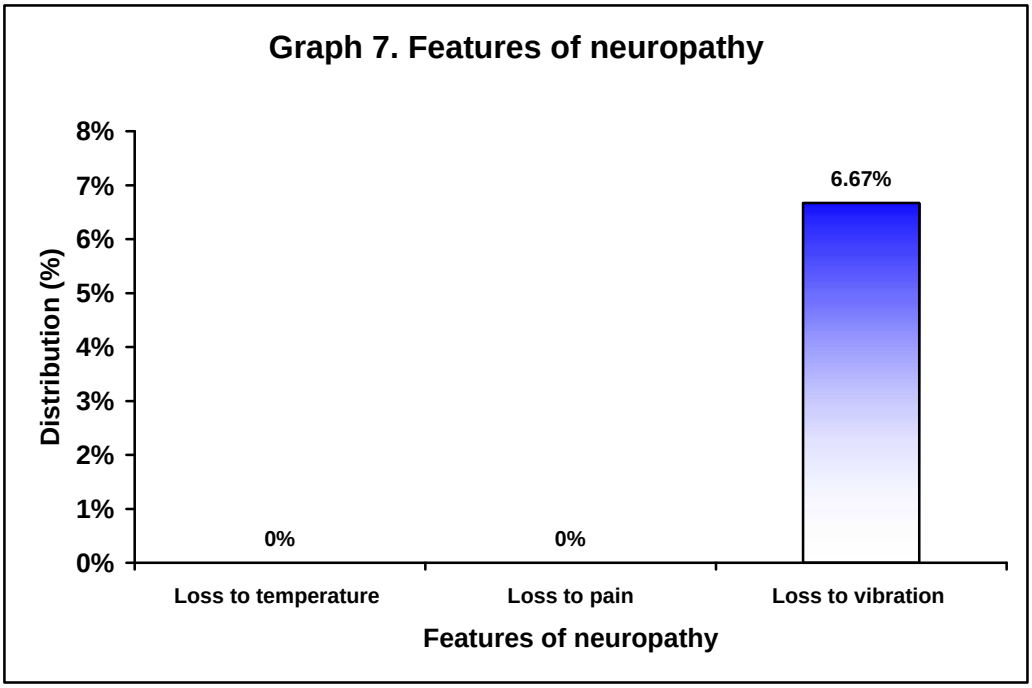
Comorbid conditions	Distribution (n=150)	
	Number	Percentage
Hypertension	39	26.00
Non diabetic kidney disease	0	0.00
Chronic diarrhoea	0	0.00
Thyroid dysfunction	0	0.00
Sepsis	0	0.00
Alcohol consumption	0	0.00



In this study 26% of the patients reported history of hypertension.

Table 7. Features of peripheral neuropathy

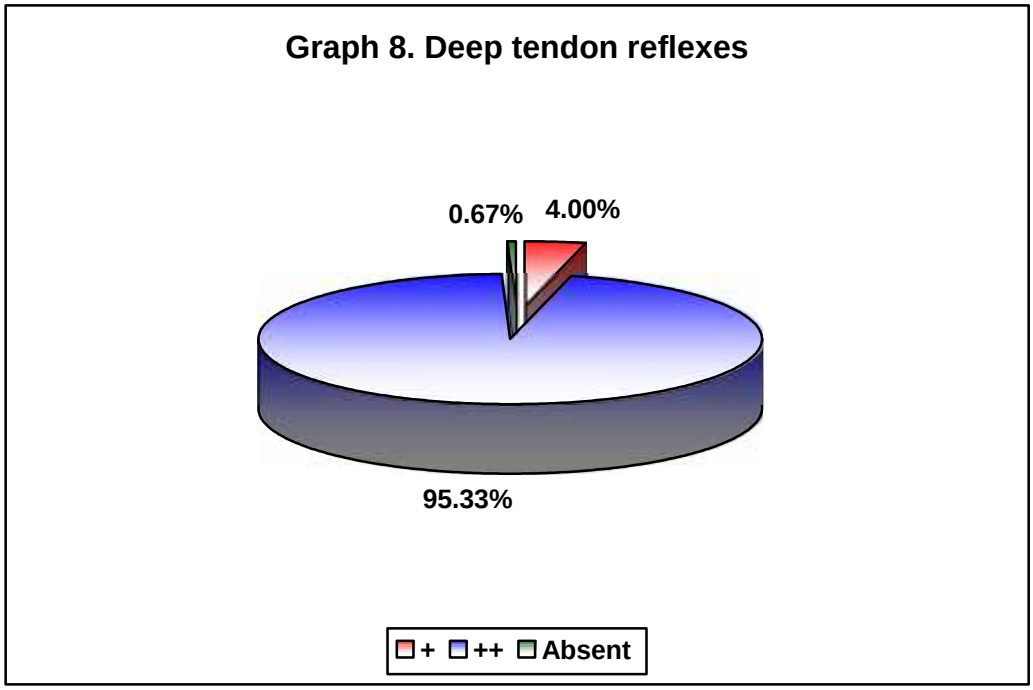
Features	Distribution (n=150)	
	Number	**Percentage**
Loss to temperature	0	0.00
Loss to pain	0	0.00
Loss to vibration	10	6.67



In the present study 6.67% of the patients had loss to vibration.

Table 8. Deep tendon reflexes

Findings	Distribution (n=150)	
	Number	**Percentage**
+	6	4.00
++	143	95.33
Absent	1	0.67
Total	**150**	**100.00**

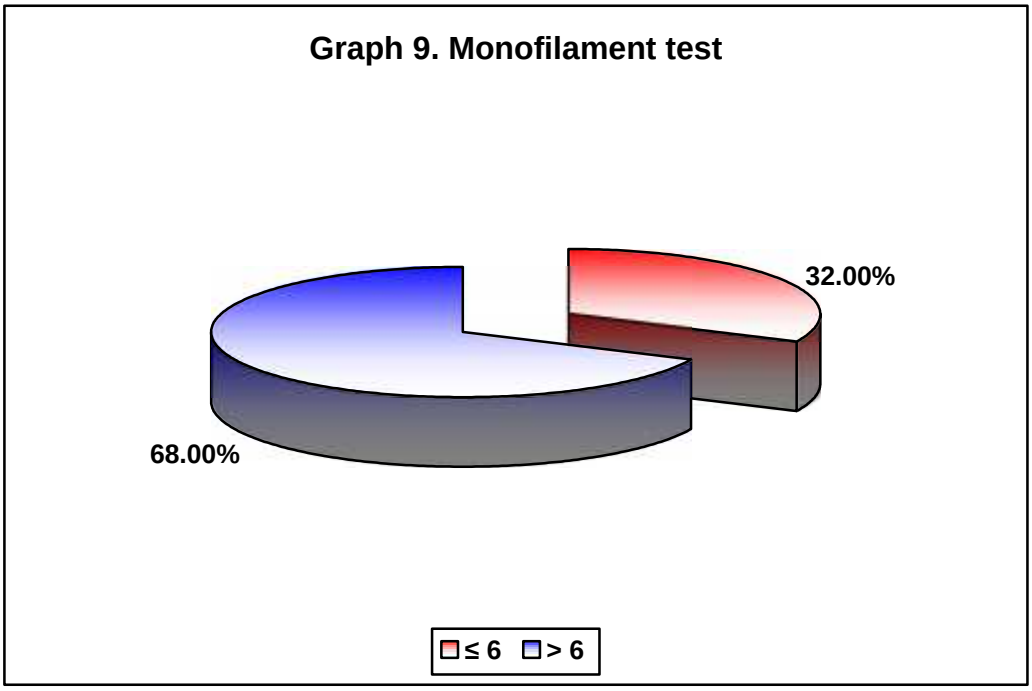


Note : +: sluggish, ++: Normal

In this study, sluggish deep tendon reflexes were present in 4% of the patients and absent reflexes were seen in 0.67%.

Table 9. Monofilament test

Findings	Distribution (n=150)	
	Number	**Percentage**
≤ 6	48	32.00
> 6	102	68.00
Total	**150**	**100.00**

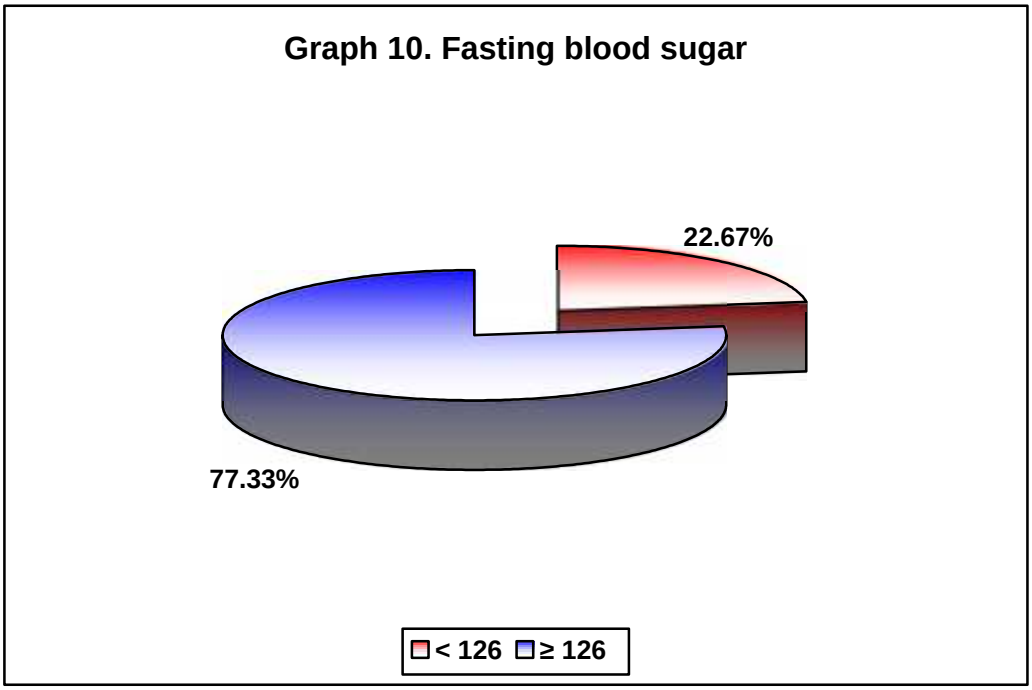


Note: >6: Normal, ≤6: Abnormal

In the present study, monofilament test showed an abnormal score of ≤ 6 in 32% of the patients.

Table 10. Fasting blood sugar

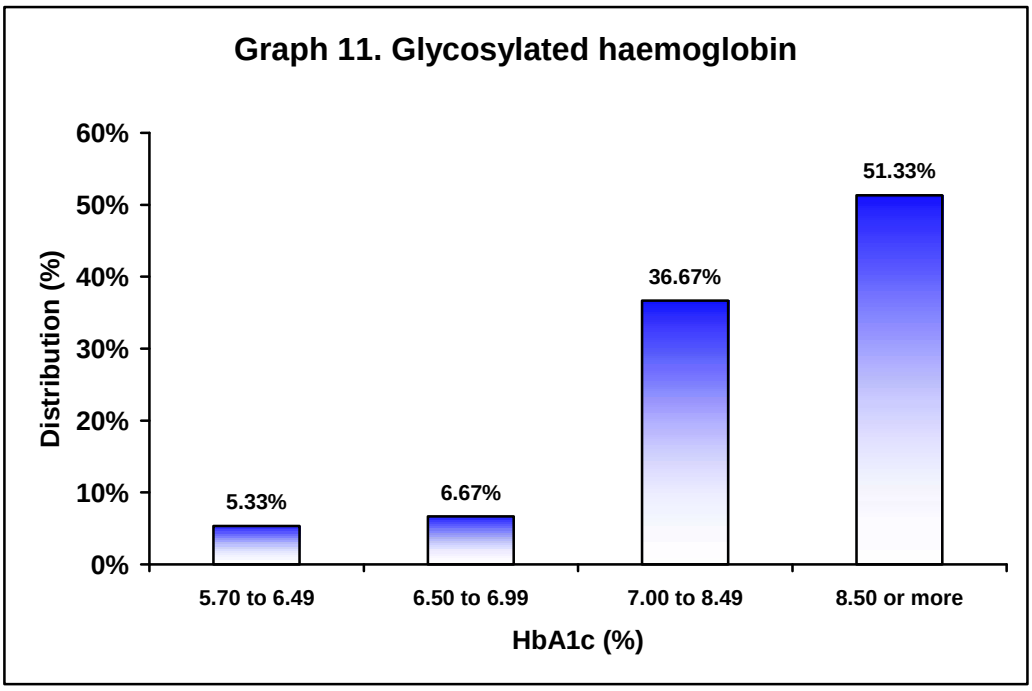
Fasting blood sugar (mg/dL)	Distribution (n=150)	
	Number	**Percentage**
< 126	34	22.67
126 or more	116	77.33
Total	**150**	**100.00**



In this study fasting blood sugar levels were of the diabetic range i.e ≥ 126 mg/dL in 77.33% of the patients.

Table 11. Glycosylated haemoglobin

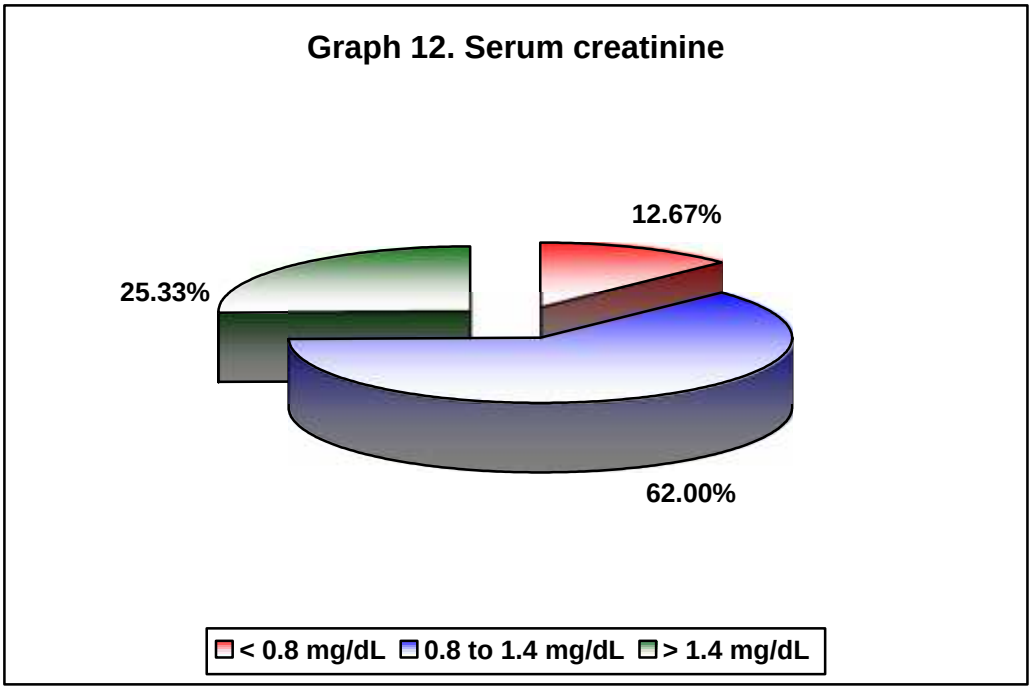
HbA1c (%)	Distribution (n=150)	
	Number	**Percentage**
5.70 to 6.49	8	5.33
6.50 to 6.99	10	6.67
7.00 to 8.49	55	36.67
8.50 or more	77	51.33
Total	**150**	**100.00**



In the present study HbA1c levels were found to be ≥ 8.5% in 51.33% of the patients while in 36.67% of the patients HbA1c levels were between 7.00 to 8.49%.

Table 12. Serum creatinine

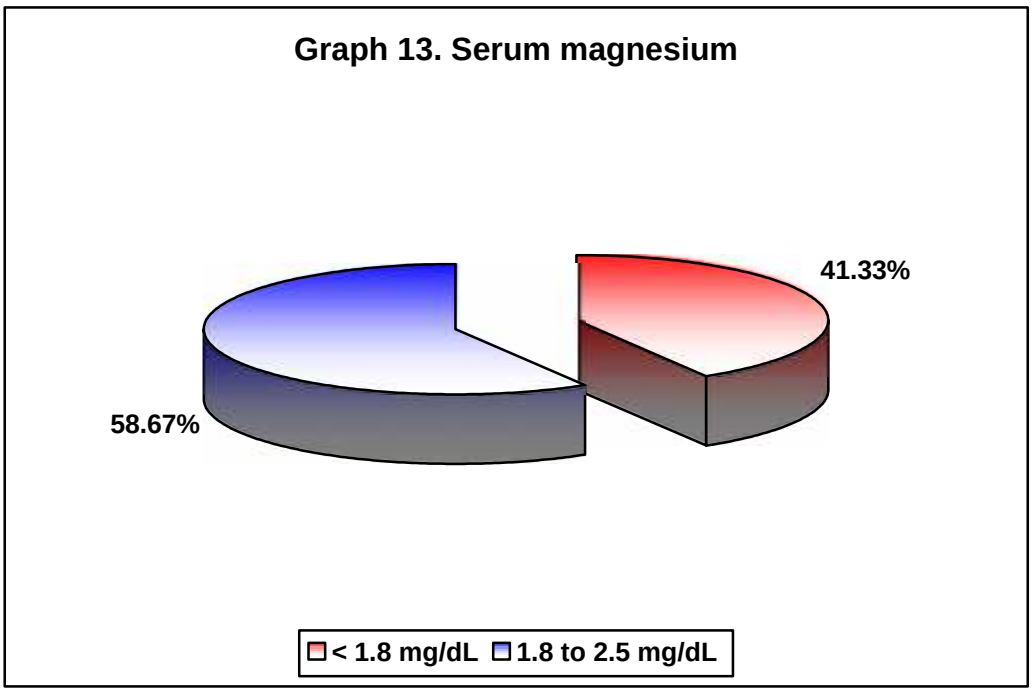
Serum creatinine (mg/dL)	Distribution (n=150)	
	Number	**Percentage**
< 0.8	19	12.67
0.8 to 1.4	93	62.00
> 1.4	38	25.33
Total	**150**	**100.00**



In the present study serum creatinine levels were > 1.4 mg/dL among 25.33% of the patients.

Table 13. Serum magnesium

Serum magnesium (mg/dL)	Distribution (n=150)	
	Number	Percentage
<1.8	62	41.33
1.8 to 2.5	88	58.67
Total	**150**	**100.00**

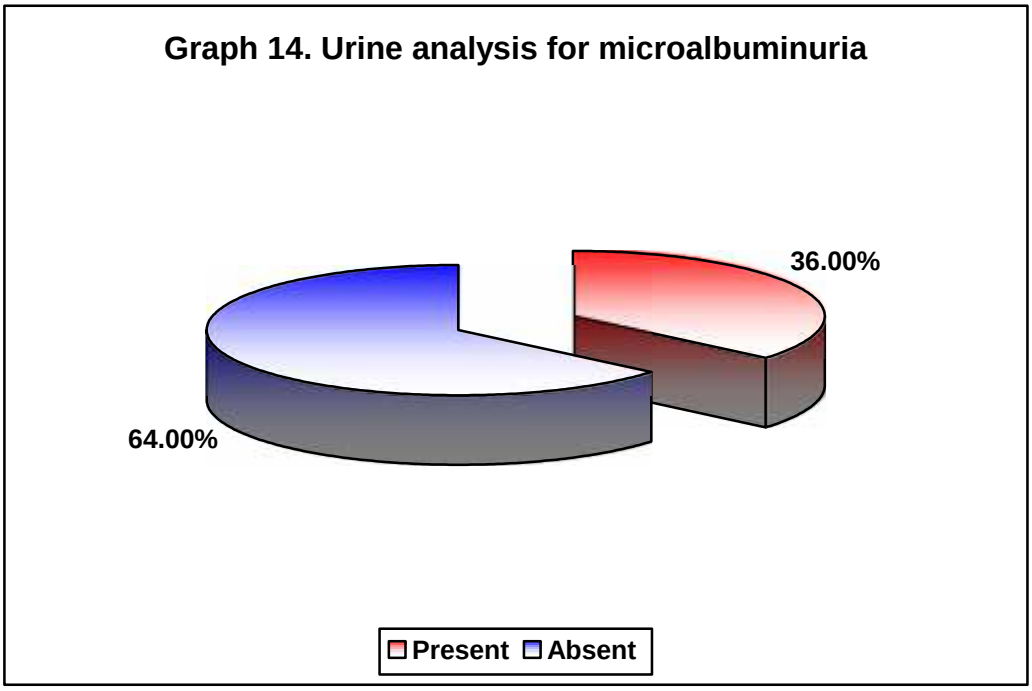


1.8-2.5mg/dL: Normal, <1.8mg/dL: Hypomagnesemia

In this study serum magnesium levels were between 1.8 to 2.5 mg/dL (normomagnesemia) among 58.67% of the patients while 41.33% of the patients had serum magnesium levels of < 1.8 mg/dL (hypomagnesemia)

Table 14. Urine analysis for microalbuminuria

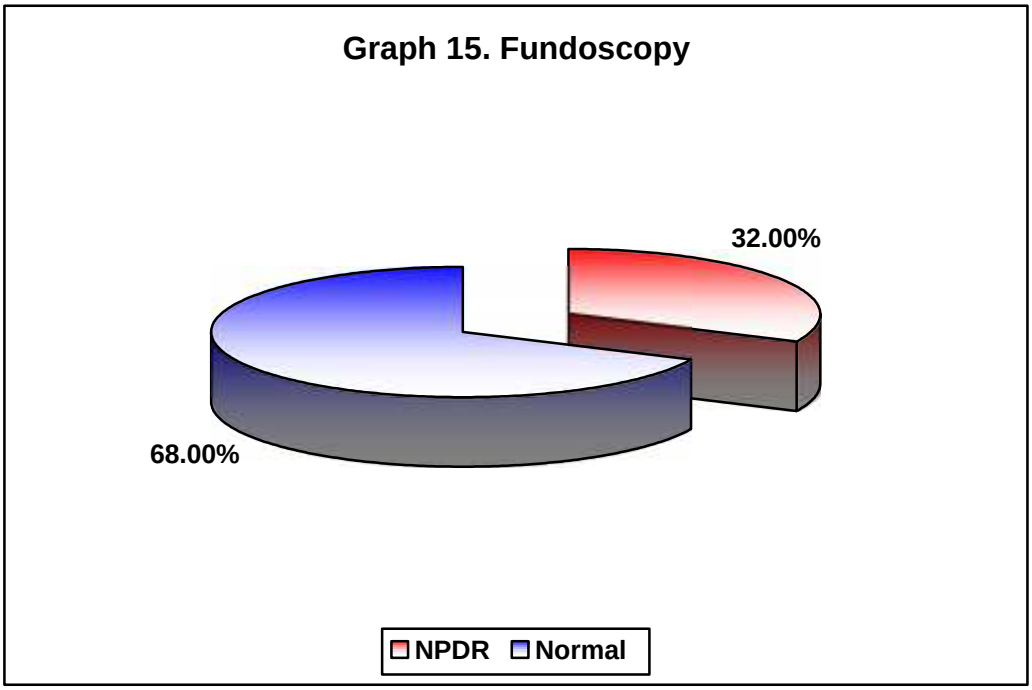
Microalbuminuria	Distribution (n=150)	
	Number	**Percentage**
Present	54	36.00
Absent	96	64.00
Total	**150**	**100.00**



In the present study based on urine analysis, microalbuminuria was present in 36% of the patients.

Table 15. Fundoscopy

Findings	Distribution (n=150)	
	Number	**Percentage**
Normal	102	68.00
NPDR	48	32.00
Total	**150**	**100.00**

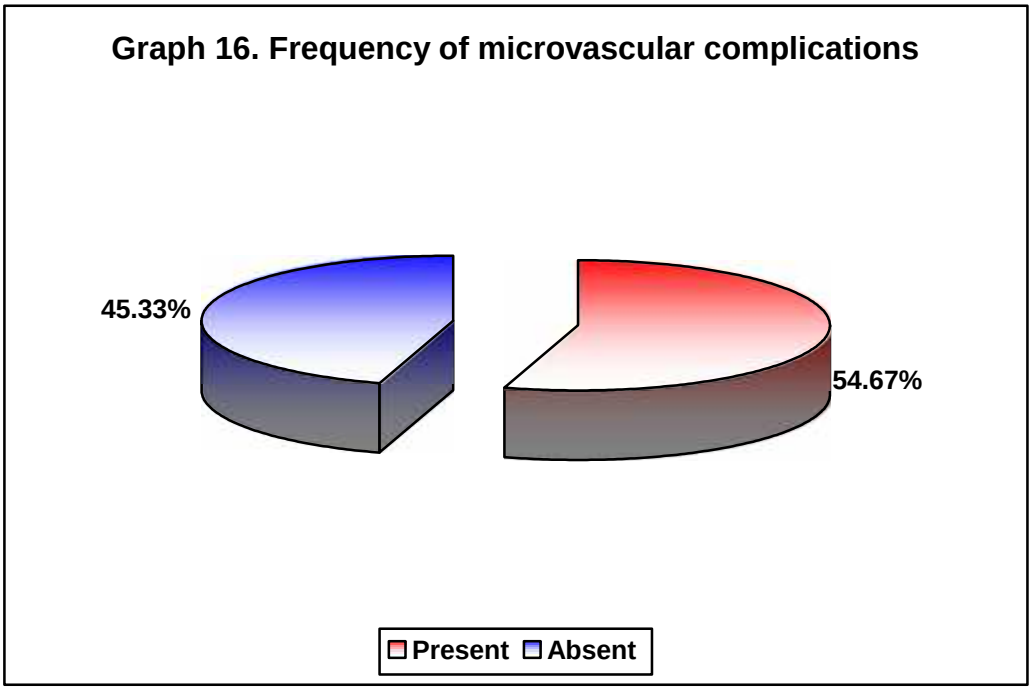


In this study fundoscopy examination revealed NPDR in 32% of the patients.

Table 16. Frequency of microvascular complications

Complications	Distribution (n=150)	
	Number	**Percentage**
Present	82	54.67
Absent	68	45.33
Total	**150**	**100.00**

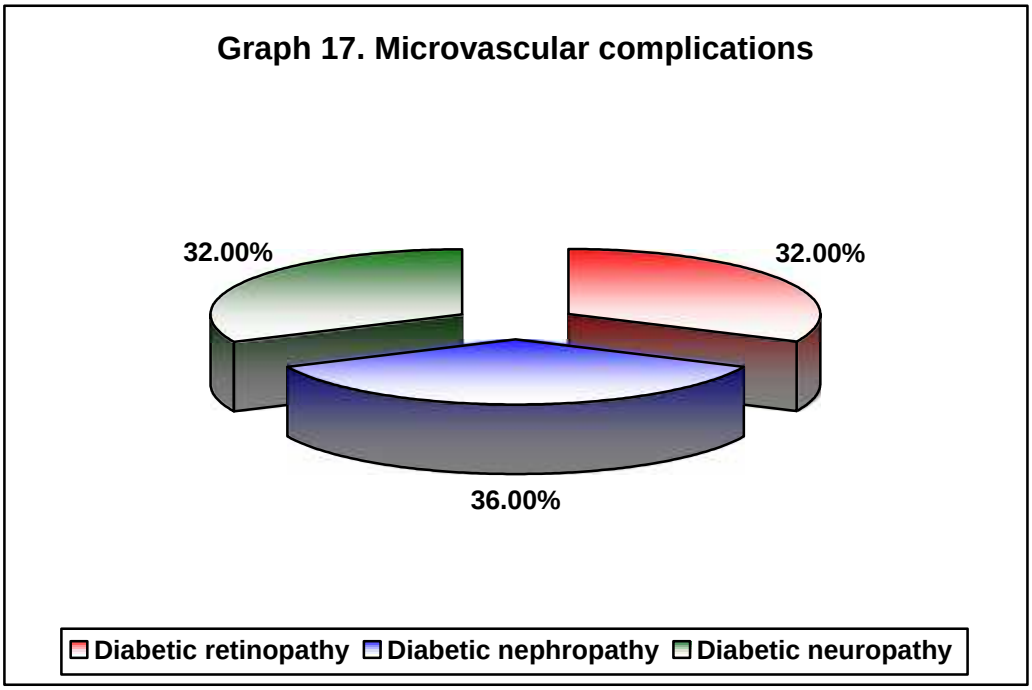
Graph 16. Frequency of microvascular complications



In the present study microvascular complications were present in 54.67% of the patients.

Table 17. Microvascular complications

Complications	Distribution (n=150)	
	Number	**Percentage**
Diabetic retinopathy	48	32.00
Diabetic nephropathy	54	36.00
Diabetic neuropathy	48	32.00



In this study the diabetic retinopathy and neuropathy were present in 32% of the patients each while diabetic nephropathy was noted in 36% of the patients.

Table 18. Characteristics of the study population

Parameters	Mean		Median	Range	
	Mean	SD		Minimum	Maximum
Temperature (0F)	98.60	0.00	98.60	98.60	98.60
Pulse rate (/Minute)	79.14	6.36	78.00	50.00	110.00
Respiratory rate (/ Minute)	15.31	2.19	16.00	12.00	26.00
Systolic BP (mm Hg)	126.19	9.38	130.00	100.00	150.00
Diastolic BP (mm Hg)	83.37	7.69	80.00	60.00	90.00
Fasting blood sugar (mg/dL)	172.80	57.84	166.00	72.00	387.00
Post prandial blood sugar (mg/dL)	238.86	78.30	218.50	116.00	516.00
HbA1c (%)	9.53	2.67	8.60	6.20	17.60
Blood urea (mg/dL)	38.57	28.76	29.00	6.00	170.00
Serum creatinine (mg/dL)	1.46	1.19	1.10	0.36	9.00
Serum magnesium (mg/dL)	1.80	0.30	1.80	1.00	2.40
Monofilament test score	8.21	2.32	10.00	3.00	10.00

The clinical and biochemical profile of the study population is as shown in table 18.

Table 19. Association of serum magnesium levels with microvascular complications

Microvascular complications	Serum magnesium (mg/dL)				Total	
	< 1.8		1.8 to 2.5			
	No.	%	No	%	No	%
Present	48	58.54	34	41.46	82	100.00
Absent	14	20.59	54	79.41	68	100.00
Total	**62**	**41.33**	**88**	**58.67**	**150**	**100.00**

p<0.001

In the present study hypomagnesemia (< 1.8 mg/dL) is seen in 58.54% of the patients with microvascular complications compared to 20.59% of the patients. This difference was statistically significant (p<0.001).

Table 20. Association of serum magnesium levels with diabetic retinopathy

Diabetic retinopathy	Serum magnesium (mg/dL)				Total	
	< 1.8		1.8 to 2.5			
	No.	%	No	%	No	%
Present	31	64.58	17	35.42	48	100.00
Absent	31	30.39	71	69.61	102	100.00
Total	**62**	**41.33**	**88**	**58.67**	**150**	**100.00**

p<0.001

In this study significantly higher number of patients with serum magnesium levels < 1.8 mg/dL had diabetic retinopathy (64.58%; p<0.001).

Table 21. Association of serum magnesium levels with diabetic nephropathy

Diabetic nephropathy	Serum magnesium (mg/dL)				Total	
	< 1.8		1.8 to 2.5			
	No.	%	No	%	No	%
Present	29	53.70	25	46.30	54	100.00
Absent	33	34.38	63	65.63	96	100.00
Total	**62**	**41.33**	**88**	**58.67**	**150**	**100.00**

p=0.017

In the present study frequency of diabetic nephropathy was significantly high in patients with serum magnesium levels < 1.8 mg/dL (53.7%; p=0.017).

Table 22. Association of serum magnesium levels with diabetic neuropathy

Diabetic neuropathy	Serum magnesium (mg/dL)				Total	
	< 1.8		1.8 to 2.5			
	No.	%	No	%	No	%
Present	38	79.17	10	20.83	48	100.00
Absent	24	23.53	78	76.47	102	100.00
Total	**62**	**41.33**	**88**	**58.67**	**150**	**100.00**

p<0.001

In this study diabetic neuropathy was present in 48 patients. Of this 79.17% had serum magnesium levels <1.8 mg/dL (hypomagnesemia) and 20.83% had same between 1.8 to 2.5 mg/dL (normomagnesemia). This difference was statistically significant (p<0.001).

Table 23. Association of serum magnesium with monofilament test (objective neuropathy)

Monofilament test	Serum magnesium (mg/dL)				Total	
	< 1.8		1.8 to 2.5			
	No.	%	No	%	No	%
6 or less	38	79.17	10	20.83	48	100.00
> 6	24	23.53	78	76.47	102	100.00
Total	**62**	**41.33**	**88**	**58.67**	**150**	**100.00**

p<0.001

In the present study significantly higher number of patients with monofilament test score of ≤ 6 had hypomagnesemia (79.17%; p<0.001).

Table 24. Association of serum magnesium with symptoms of neuropathy (tingling and numbness)

Tingling and numbness	Serum magnesium (mg/dL)				Total	
	< 1.8		1.8 to 2.5			
	No.	%	No	%	No	%
Yes	30	71.43	12	28.57	42	100.00
No	32	29.63	76	70.37	108	100.00
Total	**62**	**41.33**	**88**	**58.67**	**150**	**100.00**

p<0.001

In this study majority of the patients who presented with tingling and numbness (71.43%) had low serum magnesium levels (<1.8 mg/dL) (p<0.001).

Table 25. Comparison of mean serum magnesium levels with complications

Diabetic complications	Serum magnesium levels (mg/dL)						p value
	Complications			No complications			
	n	Mean	SD	n	Mean	SD	
Overall complications	82	1.70	0.31	68	1.92	0.25	**<0.001**
Diabetic retinopathy	48	1.65	0.30	102	1.86	0.28	**<0.001**
Diabetic nephropathy	54	1.70	0.27	96	1.85	0.31	**0.002**
Diabetic neuropathy	48	1.62	0.31	102	1.91	0.24	**<0.001**

Table 25 shows mean serum magnesium levels in patients with and without microvascular complications. The mean serum magnesium levels were significantly low among the patients who had complications (p<0.050).

Table 26. Association of serum magnesium levels with HbA1c

HbA1c (%)	Serum magnesium (mg/dL)				Total	
	< 1.8		1.8 to 2.5			
	No.	%	No	%	No	%
5.70 to 6.49	2	25.00	6	75.00	8	100.00
6.50 to 6.99	1	10.00	9	90.00	10	100.00
7.00 to 8.49	20	36.36	35	63.64	55	100.00
≥ 8.50	39	50.65	38	49.35	77	100.00
Total	**62**	**41.33**	**88**	**58.67**	**150**	**100.00**

p=0.040

In the present study serum magnesium levels were < 1.8 mg/dL among the 50.65% of the patients with HbA1c levels ≥ 8.5% compared to 25% of the patients with HbA1c levels of 5.70 to 6.49% and this difference was statistically significant (p=0.040).

Table 27. Comparison of mean serum magnesium levels with HbA1c

HbA1c (%)	Total number	Serum magnesium (mg/dL)	
		Mean	SD
5.70 to 6.49	8	1.99	0.30
6.50 to 6.99	10	1.93	0.25
7.00 to 8.49	55	1.84	0.26
≥ 8.50	77	1.73	0.32
F value		3.68	
p value		**0.013**	

The mean serum magnesium levels in patients with HbA1c levels ≥ 8.5% were significantly low (1.73±0.32 mg/dL) compared to patient with HbA1c levels 6.5 to 6.99% (1.93 ± 0.25 mg/dL). This difference was statistically significant (p=0.013).

Table 28. Association of serum magnesium levels with duration of diabetes

Duration of diabetes (Years)	Serum magnesium (mg/dL)				Total	
	< 1.8		1.8 to 2.5			
	No.	%	No	%	No	%
≤ 5	11	21.57	40	78.43	51	100.00
6 to 10	34	50.00	34	50.00	68	100.00
11 to 15	16	57.14	12	42.86	28	100.00
> 15	1	33.33	2	66.67	3	100.00
Total	**62**	**41.33**	**88**	**58.67**	**150**	**100.00**

p = 0.002

In the present study serum magnesium levels were low among significantly higher number of the patients (57.14%) who had duration of diabetes between 11 to 15 years (p=0.002).

Table 29. Comparison of mean serum magnesium levels with duration of diabetes

Duration (Years)	Total number	Serum magnesium (mg/dL)	
		Mean	SD
≤ 5	50	1.94	0.24
6 to 10	68	1.75	0.29
11 to 15	28	1.67	0.35
> 15	3	1.63	0.29
F value		6.705	
p value		**0.0002**	

In this study the mean serum magnesium levels significantly decreased with increase in duration of diabetes (p=0.0002).

DISCUSSION

Type 2 diabetes mellitus is one of the major global health challenges encountered in physicians practice in 21st century. The chronic complications of diabetes mellitus can be subdivided into microvascular (retinopathy, neuropathy, nephropathy) and macrovascular complications [coronary artery disease (CAD), peripheral arterial disease (PAD), cerebrovascular disease].[97]

Magnesium is the fourth most abundant cation in the human body and the second most abundant intracellular cation. Nevertheless, serum magnesium concentration though less sensitive, is a highly specific indicator of low magnesium status. In addition, serum magnesium measurement is the most readily available and widely used test for determination of magnesium status.[98]

The low serum magnesium levels in diabetics may contribute to the evolution of diabetic complications such as retinopathy, abnormal platelet function, cardiovascular disease and hypertension via reduction in the rate of inositol transport and subsequent intracellular depletion. Patients with severe diabetic retinopathy have lower magnesium levels than do diabetic patients with minimal retinal changes, which suggests that hypomagnesaemia may be a risk factor in development of diabetic retinopathy.[97]

Hypomagnesaemia in patients with type 2 diabetes mellitus is frequently under-diagnosed and under-evaluated due to its usual asymptomatic presentation. Hence, this study was aimed to evaluate the serum magnesium levels in patients with type 2 DM and further to correlate with the microvascular complications.

It is reported that, the prevalence of diabetes is higher in men than women.[20-26] The same was true in the present study as males (71.33%) outnumbered females (28.67%) with male to female ratio 2.48:1. These findings suggest higher prevalence of diabetes among males in this study which was consistent with the previous literature.[20-26]

Unlike in the West, where older persons are most affected, diabetes in Asian countries is disproportionately high in young to middle-aged adults.[20-26] However, in this study nearly half of the study population (50%) presented with age > 60 years. The next common age group was 46 to 60 (38%) followed by 31 to 45 years (12%). Further mean age of the study population was 60.38 ± 10.81 years and median age was 60.5 years with youngest patients being 36 years and oldest being 89 years. The findings show that diabetes mellitus was widely prevalent among elderly. The higher prevalence of diabetes among aged can be explained by the rise in the segment of geriatric population.[6]

In the present study 45.33% of the patients reported duration of diabetes between 6 to 10 years and mean duration was 7.43 ± 4.11 years. Most of the patients were on oral hypoglycaemic agents (61.33%). Nearly half of the study population (51.33%) had HbA1c levels of ≥ 8.5% suggesting maximum patients with poor glycaemic control. The mean duration of diabetes mellitus observed in the present study was comparable with a study by Badyal A. et al.[99] from Mullana, Ambala (Haryana) who reported duration of T2DM ranging from one month to eleven years with a mean duration of 9.91±5.06 years.

In the present study more than one fourth of the study population (42 patients) presented with tingling and numbness (28% each). The next common presentation was nocturnal pain (4%), blurring of vision (2.67%) and dysuria (2%). However, few patients (0.67% each) also presented with puffiness of face and sensory ataxia.

Serum magnesium levels

Marked magnesium deficiency has been reported in the previous studies in patients with type-2 diabetes.[62,67] However, some workers have also reported normal levels.[100]

In this study hypomagnesemia (<1.8mg/dL) was present in 41.33% of the patients and normomagnesemia (1.8 to 2.5 mg/dL) among 58.67% of the patients. These findings suggest that there was high prevalence of hypomagnesemia and every second patient was diagnosed to have hypomagnesemia.

Studies have reported incidence rates of 13.5–47.7% in diabetic subjects.[13,70] Prevalence of hypomagnesemia in type – 2 diabetics in our study was comparable to that reported by Nadler et al.[62] in type 2 diabetics attending outpatient clinics in the US. Walti MK et al.[101] also reported a prevalence of hypomagnesemia in type 2 diabetics at 37.6% versus 10.9% in nondiabetic controls in a study conducted in Zurich, Switzerland.

In contrast, recently, Dasgupta A., et al.[12] from Assam reported hypomagnesemia in only 11% of diabetics. The lower incidence of hypomagnesemia in their study was attributed to stricter exclusion criteria followed in the study.

The prevalence of hypomagnesemia in our study was comparable to the western studies,[62,101] and in contrast to the Indian studies,[12] our study had a higher prevalence of hypomagnesemia among diabetic subjects.

The reasons for the high prevalence of magnesium deficiency in diabetes are not clear, but may include increased urinary loss, due to osmotic diuresis, lower dietary intake, rampant use of loop and thiazides diuretics promoting magnesium wasting, diabetic autonomic neuropathies**,** impaired absorption of magnesium compared to healthy individuals. Sometimes frequent use of antibiotics and antifungals such as aminoglycosides and amphotericin in patients with diabetes may also contribute to renal magnesium wasting. Recently a specific tubular defect in magnesium reabsorption in thick ascending loop of Henle is postulated. This defect results in reduction in tubular reabsorption of magnesium and consequently hypomagnesemia. The reason for this tubular defect in diabetics in unclear. Insulin treatment has been shown to correct renal magnesium loss in diabetics.[102]

In the present study nearly half of the subset of patients (50.65%) with HbA1c levels ≥ 8.5% had hypomagnesemia while only one fourth of the patients (25%) with HbA1c levels of 6.5 to 6.99% had hypomagnesemia (p=0.040). Also the mean serum magnesium levels were significantly low in patients with HbA1c levels ≥ 8.5% (1.73 ± 0.32 mg/dL) and showed rising trend with decrease in HbA1c levels. The maximum mean serum magnesium levels (1.93 ± 0.25 mg/dL) were noted in patients with HbA1c levels from 6.5 to 6.99% (p=0.013). These findings hypothesize that, the serum magnesium levels tend to reduce in with poor diabetic control significantly.

Dasgupta A., et al[12] from Assam in their study found statistically poorer glycemic control in the hypomagnesemia patients as compared with the normomagnesemia patients. The Health Professionals Follow-Up Study and the Nurse's Health Study showed that subjects in the highest quintile of magnesium intake had a 33% lower risk of developing T2DM than those in the lowest quintile of magnesium intake.[103] A recent meta-analysis found that of the 13 selected studies, 9 studies showed a statistically significant inverse association between magnesium intake and diabetes risk and concluded that decreased magnesium intake is significantly associated with risk of type 2 diabetes in a dose-response manner.[104]

Hypomagnesemia is reported to be both a cause and result of poor glycemic control. Magnesium is a cofactor in both glucose transporting mechanisms of cell membrane and various enzymes important in carbohydrate oxidation.[17] In addition, magnesium deficiency has been shown to promote insulin resistance in multiple studies. Nadler et al.[105] have reported that insulin sensitivity decreases even in nondiabetic individuals after induction of magnesium deficiency. Like wise, elderly subjects were shown to have improved glucose tolerance when they received magnesium supplements. Thus hypomagnesaemia by itself results in poor glycemic control.

It is clear that T2DM progresses with duration of disease. In this study maximum patients (57.14%) with 10 to 15 years duration of diabetes had low serum magnesium levels ($p=0.002$). Also, the mean serum magnesium levels significantly decreased with increase in duration of diabetes (1.67 ± 0.35 mg/dL; $p=0.0002$). These findings pose strong relationship between duration of diabetes and hypomagnesemia and suggest that the patients with longer duration of diabetes are

likely to develop hypomagnesemia. Similar findings were reported in a study by Dasgupta A., et al.[12] from Assam where the mean duration of diabetes in the patients with hypomagnesemia was 6.8 years. Haquea et al.[106] found a mean diabetic duration of 8.85 years in hypomagnesemic patients and concluded that serum magnesium level has no direct relationship with diabetic duration if the diabetes is well controlled.

Serum magnesium levels and microvascular complications

Diabetic Nephropathy

In this study, microalbuminuria was present in 36% of the patients. Hence, based on this, 54 patients (36%) were found to have diabetic nephropathy.

Our observations revealed a definite association between diabetic nephropathy and lower serum magnesium levels. 53.70% of patients with diabetic nephropathy had hypomagnesemia. There was a significant difference in prevalence of hypomagnesemia in diabetics with, and without nephropathy. (p=0.017)

A study by Sajjan NB et al.[107] from Gulbarga Karnataka reported that serum levels of Magnesium showed statistically significant difference when compared in healthy subjects & subjects with Diabetic nephropathy. Recently, Dasgupta A., et al from Assam reported that, both microalbuminuria and macroalbuminuria were found at a higher incidence in the hypomagnesemia group compared with the normomagnesemia group. Corsonello et al.[84] demonstrated that diabetic patients with microalbuminuria or clinical proteinuria showed a significant decrease in serum ionized magnesium compared with normoalbuminuria group.

One of the potential pathophysiological mechanisms linking serum Mg to microalbuminuria is an amplification of insulin resistance. It is said that low serum Mg plays an important role in the pathogenesis of insulin resistance. Mg can function as a mild, natural calcium antagonist. Hence, the level of intracellular calcium is increased in Mg deficiency subjects. This increased intracellular calcium may compromise the insulin responsiveness of adipocytes and skeletal muscles leading to the development of insulin resistance which affects the tubular absorption of magnesium.[97]

Other hypothesis such as oxidative stress is becoming increasingly recognized as an important causative factor for microalbuminuria. Mg has been reported to possess antioxidant property. Hence, oxidative stress may be one of the mechanisms that underlie the association between low serum Mg and microalbuminuria.[94]

Diabetic Retinopathy

In this study, Non proliferative diabetic retinopathy was present in 32% of the patients. It was noted that out of 48 patients with retinopathy, a significantly high number of patients i.e 34 patients (64.8%) had hypomagnesemia ($p<0.001$) There was a significant difference in prevalence of hypomagnesemia in diabetics with, and without retinopathy

Recently, Dasgupta A., et al.[12] from Assam reported higher incidence of retinopathy in the hypomagnesemia group (64% vs 45.8%). The existence of a close relationship between impaired magnesium balance and retinopathy was established by Fujii et al.,[108] who found a marked depletion in plasma and erythrocyte

magnesium levels in diabetic patients with advanced retinopathy. A study from Brazil with type 1 and type 2 diabetics, however, did not demonstrate a significant correlation between the severity of retinopathy and Mg concentration in the plasma.[109]

Although the theory of depletion in the rate of inositol transport has been proposed by Grafton et al.[17] as a possible mechanism to explain the association between diabetic retinopathy and hypomagnesemia, the exact reason remains obscure.

Diabetic Neuropathy

The prevalence of DPN is generally is estimated to be 10% to 50% in patients with T2DM, and the incidence increases with age and duration of DM.[110,11]1 A nationwide survey[112] performed in 2006 by the Committee of the Korean Diabetes Association on the Epidemiology of Diabetes Mellitus (*n*=5,652) showed that the prevalence of DPN defined by neurologic symptoms or nerve conduction velocity abnormalities was 44.7%. Similarly, in the present study, a subjective evidence of neuropathy in the form of symptoms like tingling and numbness was seen in 42 patients (28%). Clinical examination revealed loss to vibrations in only 6.67% of the patients while the monofilament test showed score of ≤ 6 in 32% of the patients. Based on these assessments diabetic neuropathy was present in 36% of the patients i.e 48 patients.

In the present study a definite association between diabetic neuropathy and lower serum magnesium levels with significant difference in the prevalence of hypomagnesemia among the patients with and without neuropathy was noted. Out of

the 48 patients with neuropathy, a significantly high number of patients (38 patients) i.e 79.17% pf the patients had hypomagnesemia. In contrast Dasgupta A., et al.[12] from Assam reported that, neuropathy was comparable in both groups (82.35% vs 82.70%).

Very few studies have found that intracellular magnesium levels are lower in patients with diabetic peripheral neuropathy.[82] Most studies have reported a comparable presence of neuropathy in patients with hypomagnesemia and normomagnesemia. In contrast, our study revealed a significantly high prevelance of hypomagnesemia in patients of diabetic neuropathy.

Since there are not many studies defining the association of hypomagnesemia with diabetic neuropathy, we studied the serum magnesium levels in patients having isolated neuropathy without the evidence of other two microvascular complications (nephropathy or retinopathy). We found that isolated neuropathy was present in 14 of the patients with microvascular complications. Hypomagnesemia was seen in 11 of those 14 patients (78.57%) i.e in a significantly high percentage of patients. Among these 11 patients with hypomagnesemia, 6 of the patients had an HbA1c of <8.5% (54.54%). Hence, hypomagnesemia could be an additional independent variable determining its association with the development of neuropathy in diabetes mellitus and there is a need to validate further large scale studies to define this association.

Magnesium is known to be necessary for nerve conduction. Deficiency of magnesium increases insulin resistance which is known to affect nerve conduction. This could be one of the mechanisms to define the association of hypomagnesemia

and neuropathy in our study. However, the exact mechanism associating hypomagnesemia with neuropathy remains obscure and needs further investigations.[97]

In the present study, the overall microvascular complications were significantly high in patients with hypomagnesemia that is, 58.54% of the patients with hypomagnesemia (<1.8 mg/dL) had microvascular complications compared to 41.46% of the patients with normomagnesemia ($p<0.001$). Further, the frequency of diabetic nephropathy (53.7%) diabetic neuropathy (79.17%) and diabetic retinopathy (64.58%) was significantly high among the patients with hypomagnesemia ($p<0.050$). The mean serum magnesium levels were significantly low (1.70 ± 0.31 mg/dL) in patients who presented with microvascular complications compared to diabetic patients without having microvascular complications (1.92 ± 0.25 mg/dL) ($p<0.001$) and similar trend was noted in patients with the individual complications.

This study reveals a strong association between hypomagnesemia and microvascular complications. Hence it could be suggested that routine surveillance for hypomagnesemia is done in patients of type 2 diabetes mellitus

This study had certain limitations. As the study focused on incidence of hypomagnesemia and microvascular complications, other confounding variables such as demographic characteristics, clinical profile and biochemical profile could not be ascertained to the effect of hypomagnesemia on microvascular complications. Hence, further studies considering these confounding variables will focus higher accuracy of relationship between hypomagnesemia and microvascular complications in T2DM.

CONCLUSION

Hypomagnesemia is widely prevalent (41.33%) among patients with type 2 diabetes mellitus and lower serum magnesium were seen in patients with poor control and longer duration of diabetes.

There was a strong relationship between hypomagnesemia and microvascular complications (diabetic retinopathy, nephropathy and neuropathy)

With special reference to neuropathy, there was a higher prevalence of hypomagnesemia in neuropathy than with other complications.

Low serum magnesium is one of the additional risk factors for the development of microvascular complications in type 2 diabetes mellitus.

SUMMARY

There is high frequency of hypomagnesaemia in patients with type 2 diabetes mellitus and it may lead to several microvascular complications. This study was aimed to evaluate the serum magnesium levels in patients with type 2 DM and further to correlate with microvascular complications.

The present one year hospital based cross-sectional study was done on 150 patients with type 2 diabetes mellitus from January 2014 to December 2014 in Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. All the patients were investigated for serum magnesium levels and microvascular complications.

In the present study majority of the patients (71.33%) were males and male to female ratio was 2.48:1. The commonest age group was > 60 years (50%) and the mean age was 60.38 ± 10.81 years. Most of the patients presented with features of tingling and numbness (28%). The duration of diabetes in 45.33% of the patients was between 6 to 10 years and mean duration was 7.43 ± 4.11 years. Fasting blood sugar levels were ≥ 126 mg/dL in 77.33% of the patients and HbA1c levels were ≥8.5% in 51.33% of the patients. Serum magnesium levels were < 1.8 mg/dL in 41.33% of the patients. Microvascular complications were present in 54.67% of the patients and diabetic retinopathy was noted in 32% of the patients while diabetic nephropathy and diabetic peripheral neuropathy were present in 36% of the patients each. Low serum magnesium levels had a strong association with diabetic retinopathy ($p<0.001$), nephropathy ($p=0.017$) and peripheral neuropathy ($p<0.001$).

Furthermore, serum magnesium levels were found to be low in patients with poor glycaemic control (p=0.040) and higher duration of diabetes (p=0.002).

Hypomagnesemia is widely prevalent in patients with type 2 diabetes mellitus and also associated with poor glycaemic control and longer duration. In addition to duration of diabetes and control, hypomagnesemia is an additional independent risk factor for the development of microvascular complications.

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ANNEXURE I – CONSENT FORM

TITLE OF RESEARCH STUDY: SERUM MAGNESIUM LEVELS IN TYPE 2 DIABETES MELLITUS AND ITS ASSOCIATION WITH THE MICROVASCULAR COMPLICATIONS.

Principal Investigator:
Dr. ** *********
Post Graduate Student,
Department of General Medicine,
Jawaharlal Nehru Medical College,
Belgaum.

Guide:
Dr. **** ************
Associate Professor,
Department of General Medicine,
Jawaharlal Nehru Medical College,
Belgaum.

Introduction and Purpose

Hypomagnesemia is known to occur at an increased frequency in diabetics, But it has not been included as a routine screening in diabetics. Hence, this study has been taken up to screen the type 2 diabetics admitted to our hospital for hypomagnesemia and evaluate the association of S. Magnesium levels with the microvascular complications.

Procedure

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

Risk and Benefits

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

You may not be directly benefited from the study but this study will help to correlate magnesium levels with diabetic complications and would possibly help to reduce the complications in future.

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 in the study, you can withdraw from the study at any time during the study if you wish to. Your decision will not change the present or future health care or other services that you receive. The study doctor or sponsor may stop your participation in this study at any time. If you choose not to take part in the study, you will still receive the standard treatment for patients with your condition.

Privacy and Confidentiality

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

Institution / Sponsor's policy

In the event of injury related to the study, treatment will be made available at KLEs Prabhakar Kore Hospital and Medical Research centre. There is no compensation or payment for such medical treatment by law.

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.

Queries

In case of any queries during study or in future you may contact following persons,

1. Dr. ****** *******, Investigator, PG in General Medicine, JNMC, Belgaum. Phone no. ****** ******.
2. Dr. ****** ******** Associate Professor, Dept of General Medicine, JNMC, Belgaum. Phone no. ***** ******
3. Dr. Chairperson, JNMC Ethical committee for Human research. Phone no. ***** *****.

Consent Statement

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

Name of the Participant: _____

Signature / Thumb print:_____

Name of the Witness: _____

Signature/ Thumb print:_____

Investigator Name: _____

Signature: _____

Date:

Place:

ANNEXURE II – PROFORMA

TITLE: "SERUM MAGNESIUM LEVELS IN TYPE 2 DIABETES MELLITUS AND ITS ASSOCIATION WITH THE MICROVASCULAR COMPLICATIONS"

Case No. :

Name : Age /

Sex :

In Patient Number :

Address :

Occupation :

Complaints at presentation

History of diabetic complications

Visual symptoms

Blurring of vision

Progressive loss of vision

Sudden blindness

Renal symptoms

Dysuria

Flank pain

Puffiness of face

Symptoms of neuropathy

Tingling and numbness

Nocturnal pains

Trophic ulcers

Sensory ataxia

Charcot's joints

Wrist drop

Foot drop

Symptoms of radiculopathy

Symptoms of autonomic neuropathy

Dysphagia

Diarrhea

Vomiting

Syncope

History of diabetes

Duration of diabetes

Family history

Treatment taken

Past history

Personal history

Diet

Appetite

Sleep

Bowel and bladder habits

Other habits

General physical examination

Pallor : Yes / No

Icterus : Yes / No

Lymphadenopathy : Yes / No

Cyanosis Yes / No

Clubbing : Yes / No

Edema : Yes / No

Vitals

Temperature :

Pulse :

Respiratory rate :

Blood Pressure :

Systemic examination

Respiratory System :

Cardiovascular System :

Per Abdomen :

Central Nervous System :

Monofilament test :

Fundoscopy :

Reflexes :

Loss to temperature :

Loss to vibration :

Loss to pain :

Diagnosis

Investigations

Fasting blood sugar

Post Prandial blood sugar

HbA1c

S. Magnesium

Blood urea

S. Creatinine

Urine routine and microscopy

Microalbuminuria

Hemoglobin

Total count

Differential count

Fasting Lipid profile

Thyroid function

USG Abdomen

ANNEXURE III – KEY TO MASTER CHART

-	-	Absent
+	-	Present
BP	-	Blood Pressure
HbA1c	-	Glycosylated haemoglobin
I	-	Insulin
mg/dL	-	Milligrams per deciliter
Mm Hg	-	Millimeters of mercury
N	-	No
n	-	Normal
ND	-	Newly detected
NPDR	-	Non proliferative diabetic retinopathy
NVBS	-	Normal vesicular breath sounds
OHAs	-	Oral hypoglycaemic agents
Y	-	Yes