
Research Article

Study of Relation between Serum Magnesium Levels and Vascular Complications in Patients of Type 2 Diabetes Mellitus

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Abstract:

Vascular complications in a patient with Diabetes have been studied for many decades. Many hypotheses have been proposed and some have evidence based findings. One such hypothesis is associated with serum magnesium levels. Magnesiuria is well known in diabetics and the role of magnesium in endothelial function is well established. Here we studied the incidence of vascular complications in diabetics and correlated with serum magnesium levels.

Key Words: Diabetes mellitus, Magnesium, Vascular complications.

Introduction

Diabetes is a growing public health burden across the world, particularly in the developing countries.¹ The World Health Organization estimates that more than 180 million people worldwide have diabetes, and by 2030 it is expected that this number will have doubled. According to Diabetes Atlas published by the International Diabetes Federation (IDF), there were an estimated 40 million persons with diabetes in India in 2007 and this number is predicted to rise to almost 70 million people by 2025. The countries with the largest number of diabetic people will be in India, China and USA by 2030. The term Diabetes mellitus, derived from Greek words meaning *siphon* and *sweet*, is a heterogeneous group of metabolic disorders characterized by chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism resulting 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.² Depending on aetiology of the diabetes, factors contributing to hyperglycaemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production.³ In some cases the primary defect is the synthesis, release or action of insulin; in other instances a metabolic defect beyond insulin is responsible. In diabetes chronic elevation of blood glucose level leads to damage of blood vessel (angiopathy). This is because endothelial cells lining the blood vessels take in more glucose than normal, as they do not depend on insulin.⁴ The excess glucose causes non-enzymatic glycosylation of proteins and accumulation of polyol such as sorbitol, these end products of the advanced glycosylation, cause irreversible changes and this process culminates in cellular damage. This AGEs trigger dyslipidaemia and hypertension, the chronic hyperglycaemia that results may eventually lead to dysfunction, especially the heart, kidneys, blood vessels, nerves and eyes.⁵ Besides multiplying the risks of coronary heart disease, diabetes enhances the incidence of cerebrovascular strokes. Moreover it is the leading cause of acquired blindness and accounts for over 25 percent of cases with end stage renal failure as well as 50 percent of non-traumatic lower limb amputations.

Several vitamins and minerals act as cofactors in an enzyme reaction regulated by insulin. Deficiencies of certain vitamins and minerals such as vitamin E, potassium, magnesium, zinc and chromium may aggravate carbohydrate intolerance. Out of all these, it is relatively easy to detect potassium or magnesium concentrations in serum and to replace them based on their low serum levels.⁶ Homeostasis of the trace elements such as zinc, copper, iron and magnesium (Mg) has been found to play an important role in the pathogenesis of diabetes and diabetic complications.⁷ Magnesium is an essential element and has a fundamental role in carbohydrate metabolism in general and in insulin action in particular. The concentration of magnesium in serum of healthy people is constant. In 25 to 39% of people with diabetes, low concentrations of serum magnesium. 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.⁸

Mg has received considerable attention for its potential in improving insulin sensitivity and preventing diabetes and its cardiovascular complications.^{9,10} However, results are inconsistent among the studies.^{11,12,13} Hypomagnesaemia is a common feature in patients with type 2 diabetes. Although diabetes can induce hypomagnesaemia, magnesium deficiency has also been proposed as a risk factor for type 2 diabetes. Magnesium is a necessary cofactor for several enzymes that play an important role in glucose metabolism. Animal studies have shown that magnesium deficiency has a negative effect on the post-receptor signaling of insulin. On the post-receptor signalling of insulin. Some short-term metabolic studies suggest that magnesium supplementation has a beneficial effect on insulin action and glucose metabolism.¹⁴

Hypomagnesaemia has long been known to be associated with diabetes mellitus. Low serum magnesium level has been reported in children with insulin dependent diabetes mellitus and through the entire spectrum of adult type 1 and type 2 diabetes mellitus regardless of the type of therapy.¹⁵ Initially the cause of hypomagnesaemia was attributed to (1) osmotic renal losses from glycosuria (2) decreased intestinal magnesium absorption and redistribution of magnesium from plasma into red blood cells caused by insulin effect. Recently

a specific tubular magnesium defect in diabetes has been postulated.

Hypermagnesuria results specifically from a reduction in tubular absorption of magnesium.¹⁶ Magnesium is involved on multiple levels in insulin secretion, binding and activity. Cellular magnesium deficiency can alter of the membrane bound sodium-potassium-adenosine triphosphatase which is involved in the maintenance of gradients of sodium and potassium and in glucose transport.¹⁷ In diabetics there is a direct relationship between serum magnesium level and cellular glucose disposal that is independent of insulin secretion. This change in glucose disposal has been shown to be related to increased sensitivity of the tissues to insulin in the presence of adequate magnesium levels.¹⁸

Magnesium deficiency has been found to be associated with diabetic micro vascular disease. Low serum magnesium level correlated positively with the velocity of regaining basal vascular tone after hyperaemia. Hypomagnesemia has been demonstrated in patients with diabetic retinopathy, with lower magnesium levels predicting a greater risk of severe diabetic retinopathy.¹⁹ Magnesium depletion has been associated with multiple cardiovascular implications: arrhythmogenesis, vasospasm, and hypertension and platelet activity.²⁰

In elderly type 2 diabetics Paolisso et al demonstrated that oral magnesium supplements given for 4 weeks resulted in lower fasting plasma glucose levels, increased plasma and erythrocyte magnesium levels and a slight but statistically significant increase in B-cell response to glucose and arginine.²¹

Normal values of serum magnesium are between 1.7 and 2.4 mg/dl. Values below the threshold of 1.6 mg/dl are defined as hypomagnesaemia. Magnesium depletion occurs when intra cellular magnesium stores are depleted. In majority of patients decreased total magnesium is associated with low serum Mg as well. Thus in clinical practice patients with serum hypomagnesaemia are considered having intra cellular magnesium depletion.

A growing body of evidence suggests that magnesium plays a pivotal role in reducing cardiovascular risks and may be involved in the pathogenesis of diabetes. There is a substantial body of epidemiological and experimental evidence that, magnesium deficiency may contribute to the progression of atherosclerosis. So there is a need to study, whether the extracellular status of magnesium (Mg) could be a biochemical mediator between hyperglycaemia and vascular complications in diabetes mellitus.

In this study estimation of serum magnesium was carried out in patients of type 2 diabetes mellitus, with vascular complications and a correlation of these values with the metabolic control of diabetes is also studied.

Review of Literature

Definition: Diabetes mellitus is a group of metabolic disease characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycaemia of diabetes is associated with long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels.

DIABETES MELLITUS is emerging as a chronic non-communicable disease of concern in developing countries due to changing environments, urbanization and altered life-styles. Furthermore, Indians have high ethnic susceptibility for developing diabetes at a younger age group and develop vascular complications earlier and more frequently during the natural progression of the disease.

Diabetes mellitus is a metabolic disease of growing concern not only because of its adverse effect on various metabolism of the body, but also because it puts the patient at higher risk of developing various macro and microvascular complications like cardiovascular disease, cerebrovascular disease, peripheral arterial disease, retinopathy, nephropathy, neuropathy etc. Diabetes is on track to become one of the major global health challenge of the 21st century.

DIAGNOSIS OF DIABETES MELLITUS

WHO criteria²² for diabetes mellitus,

Fasting Blood Sugar ≥ 126 mg/dl or

2 hour Post Prandial ≥ 200 mg/dl or

HbA1C level ≥ 6.5 .

Criteria for the Diagnosis²³:

Normoglycemia

IFG or IGT

Diabetes mellitus

FPG < 110 mg/dl

FPG > 110 and <126 mg/dl

FPG > 126 mg/dl

2h PPBG < 140 mg/dl

2h PPBG > 140 and < 200 mg/dl

2h PPBG >200mg/dl.

Symptoms of DM and

RBS > 200 mg/dl.

A diagnosis of diabetes must be confirmed on a subsequent day by measurement of FPG, 2 hr PG or random plasma glucose (if symptoms are present). The FPG test is greatly preferred because of ease of administration, convenience, acceptability to patients and lower cost. Fasting is defined as no caloric intake for at least 8 hours.

This test requires the use of glucose load containing the equivalent of 75g anhydrous dissolved in water.

Etiologic Classification of Diabetes mellitus²⁴

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

a.Immune mediated

b.Idiopathic

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

III.Other specific types:

A Genetic defects of beta cell function

B Genetic defects in insulin action

C Disease of the exocrine pancreas

D Endocrinopathies

E Drug or Chemical related

F Infections

G Uncommon factors of immune mediated diabetes

H Genetic syndromes sometimes associated with diabetes

IV. Gestational Diabetes mellitus (GDM)

TYPE-2 DIABETES:

This form of diabetes is characterized by insulin resistance and usually relative (rather than absolute) insulin deficiency. Most patients with this form of diabetes are obese. Ketoacidosis seldom occurs spontaneously. These patients are at increased risk of developing macro vascular complications. Insulin secretion is defective in these patients and insufficient to compensate for the insulin resistance.

Genetics: Genetic factors are more important in the aetiology of type 2 than type1 diabetes. The majority of the cases of type diabetes are multifactorial in nature, with interaction of environmental and genetic factors.

Insulin Resistance:

Increased hepatic production of glucose and resistance to the action of insulin in muscle are invariable in obese and non-obese patients with type 2 diabetes. Insulin resistance may be due to any one of the three general causes: an abnormal insulin

molecule, and excessive amount of circulating antagonists or target tissue defects. The characteristic feature of type 2 diabetes is that it is often associated with other medical disorders including obesity, hypertension and hyperlipidemia.

It has been suggested that this cluster of conditions, all of which predispose to cardiovascular disease, is specific entity (the 'metabolic syndrome or syndrome X') with insulin resistance being the primary defect.

METABOLIC DISTURBANCE IN DIABETES

The hyperglycaemia of diabetes develops because of an absolute (type 1 diabetes) or a relative (type 2 diabetes) deficiency insulin, resulting in decreased anabolic and increased catabolic effects. In both type-1 and type- 2 diabetes, insulin's actions are also impaired by insensitivity of target tissues. While this is the fundamental defect in type-2 diabetes, hyperglycaemia can also induce insulin resistance through glucose toxicity.

Complications of Diabetes

I. ACUTE:

1. Hypoglycemia: Occurs often in diabetic patients treated with insulin. The risk of hypoglycemia is the most important single factor limiting the attainment of the therapeutic goal, namely near normal glycemia.

2. Diabetic Ketoacidosis: Is a major medical emergency and remains a serious cause of morbidity, principally in patients with type 1 diabetes.

The cardinal biochemical features of diabetic Ketoacidosis are

- Hyperglycaemia
- Hyperketonaemia
- Metabolic acidosis Complications:
- Cerebral edema
- ARDS
- Thromboembolism
- DIC
- Acute circulatory failure

3. Non Ketotic hyperosmolar diabetic coma: This condition is characterized by severe hyperglycaemia without significant hyperketonaemia or acidosis. Thromboembolic complications are common.

4. Lactic Acidosis: High concentration of lactic acid (> 5.0 mmol/L) in the blood.

II. CHRONIC

Chronic complications of diabetes can be classified as microvascular (due to damage to small blood vessels) and macro vascular (due to damage to larger blood vessels). Microvascular complications include damage to eyes (retinopathy) leading to blindness, to kidneys (nephropathy) leading to renal failure and to nerves (neuropathy) leading to impotence and diabetic foot disorders (which include severe infections leading to amputation). Macro vascular complications include cardiovascular diseases such as heart attack, stroke and insufficiency in blood flow to legs.

Macro vascular Disease

- Macrovascular disease (macroangiopathy) refers to atherosclerosis. Atherosclerosis is a form of arteriosclerosis (thickening and hardening of arterial walls), characterized by

plaque deposits of lipids, fibrous connective tissue, calcium, and other blood substances.

- Diabetes mellitus is a major factor for morbidity and mortality through premature and accelerate atherosclerosis. It affects the main vessels namely coronary vessels, cerebral vessels and arteries that supply blood to muscles.
- Coronary and cerebrovascular disease is 2 –4 times as common in a diabetic and the post-infarction mortality is higher.
- Peripheral vascular disease is a 4 – 6 times more common in diabetic; associated presence of neuropathy accentuates diabetic foot problems.
- The usual relative protection against atherosclerosis prior to menopause is lost in diabetic women.

PATHOGENESIS

Multiple mechanisms contribute to arterial disease in patients with type II Diabetes. A variety of risk factors converge on the artery to promote atherogenesis in individuals with type II diabetes. Skeletal muscles may be resistant of insulin action, which decreases the utilization of glucose and free fatty acids, causing hyperglycemia and increased levels of circulating free fatty acids. In the face of the insulin resistance, the pancreas initially attempts to compensate by producing more insulin, yielding hyperinsulinemia, itself a risk factor for arteriopathy. A high burden of abdominal fat presents the liver with elevated levels of free fatty acids through the portal circulation. This excess of free fatty acids will drive the overproduction of TG-rich lipoprotein particles, including VLDL. A reciprocal decrease in HDL accompanies the hypertriglyceridemia characteristic of the type II diabetic state. In addition to the increase in fasting TGs, patients with diabetes may have an accentuated response to dietary fat, yielding an exaggerated postprandial lipemia, indicated by the creamy supernatant over the plasma in the test tube. The adipocyte can also release proinflammatory cytokines such as TNF- α , which not only have direct effects on vascular wall cells that can promote atherogenesis, increased fibrinogen, and an increase in the inhibitor of fibrinolysis.

DIAGNOSIS

It is diagnosed by imaging of the affected vessel or detecting the damage produced due to the affected vessel. CT scan of the brain shows infarcted portion of brain due to atheromatous cerebral or carotid vessels. Echocardiography of heart shows infarcted myocardium which is identified by its hypokinesia. Doppler of the vessels of affected limbs shows atheromatous plaque of the affected vessel.

Micro Vascular Disease

Diabetic Nephropathy

Diabetic nephropathy (nephropatia diabetica), also known as Kimmelstiel–Wilson syndrome, or nodular diabetic glomerulosclerosis and intercapillary glomerulonephritis, is a progressive kidney disease caused by angiopathy of capillaries in the kidney glomeruli. It is characterized by nephrotic syndrome and diffuse glomerulosclerosis.

Diabetic nephropathy is usually preceded by the onset of diabetic retinopathy; the evidence of nephropathy without retinopathy gives the suspicion that the renal impairment is not caused by Diabetes itself.

Glomerular hyperfiltration is the basic pathophysiology in diabetic nephropathy. This leads to intraglomerular hypertension. Progression from glomerular hyperfiltration

leads to the stage of basement membrane thickening. This is the earliest detectable change in the course of diabetic nephropathy. This is followed by expansion of mesangium and finally by nodular sclerosis. At this stage, the kidney may leak more serum albumin (plasma protein) than normal in the urine (albuminuria), and this can be detected by sensitive medical tests for albumin. This stage is called "microalbuminuria". As diabetic nephropathy progresses, increasing numbers of glomeruli are destroyed by progressive nodular glomerulosclerosis. Consequently, urine albumin increases to the point that it may be detected by ordinary urinalysis techniques. At this stage, a kidney biopsy generally clearly shows diabetic nephropathy. The Armani-Ebstein change or Armani-Ebstein cells consists of deposits of glycogen in the tubular epithelial cells (pars straight of proximal convoluted tubule and loop of Henle). Because most DIABETICS are treated before this stage, it is very rare to see it at the present time. It appears in decompensated diabetics with glycemia higher than 500 mg/dL and in the presence of severe glycosuria; it is a reversible alteration without functional manifestations. The interstitium shows nonspecific chronic changes.

DIAGNOSIS

The first laboratory abnormality is a positive microalbuminuria test. Diagnosis is based on the measurement of urinary albumin.

It is defined as:

- Normoalbuminuria: urinary albumin excretion <30 mg/24h, it is the physiological state;
- Microalbuminuria: urinary albumin excretion in the range of 30–299 mg/24h;
- Clinical (overt) albuminuria: urinary albumin excretion ≥300 mg/24h.

Another diagnostic tool is glomerular filtration rate estimate (eGFR) based on Cockcroft and Gault or on Levey's (MDRD modified) formulae, both based on creatinine values and patient's age. Normal eGFR is above 90 mL/min/1.73 m²; different stages of renal damage can be identified by eGFR intervals.

STAGES:

1. Hyperfunction
2. Normoalbuminuria
3. Incipient Diabetic nephropathy (Microalbuminuria)
4. Overt Diabetic nephropathy
5. Uremia.

Diabetic Retinopathy

It is retinopathy (damage to the retina) caused by complications of diabetes, which can eventually lead to blindness. It is an ocular manifestation of Diabetes, a systemic disease, which affects up to 80 percent of all patients who have had diabetes for 10 years or more. Diabetic retinopathy is the result of microvascular retinal changes. Hyperglycaemia-induced intramural pericyte death and thickening of the basement membrane lead to incompetence of the vascular walls. These damages change the formation of the blood-retinal barrier and also make the retinal blood vessels become more permeable. An over accumulation of glucose and/or fructose damages the tiny blood vessels in the retina.

DIAGNOSIS

Diabetic retinopathy is diagnosed by eye examination that include,

Visual acuity test: This test uses an eye chart to measure how well a person sees at various distances (i.e., visual acuity).

Pupil dilation: The eye care professional places drops into the eye to dilate the pupil. This allows him or her to see more of the retina and look for signs of diabetic retinopathy.

Ophthalmoscopy or fundus photography: Ophthalmoscopy is an examination of the retina by (1) slit lamp bio microscope with a special magnifying lens that provides a narrow view of the retina, or (2) indirect ophthalmoscope with a bright light, and a special magnifying gives a wide view of the retina, or (3) Hand-held ophthalmoscopy. Fundus photography generally recreate considerably larger areas of the fundus, and has the advantage of photo documentation for future reference, as well as availing the image to be examined by a specialist at another location and/or time.

Fundus Fluorescein angiography (FFA): This is an imaging technique which relies on the circulation of Fluorescein dye to show staining, leakage, or non-perfusion of the retinal and choroidal vasculature.

STAGES

1. Mild Nonproliferative Retinopathy.
2. Moderate Nonproliferative Retinopathy.
3. Severe Nonproliferative Retinopathy.
4. Proliferative Retinopathy.

Diabetic neuropathy

These conditions are thought to result from Diabetic microvascular injury involving small blood vessels that supply nerves (vasa nervorum) in addition to macrovascular conditions that can culminate in Diabetic neuropathy. Relatively common conditions which may be associated with Diabetic neuropathy include third nerve palsy; mononeuropathy; mononeuropathy multiplex; diabetic amyotrophy; a painful polyneuropathy; autonomic neuropathy; and thoracoabdominal neuropathy.

Vascular and neural diseases are closely related and intertwined. Blood vessels depend on normal nerve function, and nerves depend on adequate blood flow. The first pathological change in the microvasculature is vasoconstriction. As the disease progresses, neuronal dysfunction correlates closely with the development of vascular abnormalities, such as capillary basement membrane thickening and endothelial hyperplasia, which contribute to diminished oxygen tension and hypoxia. Neuronal ischemia is a well-established characteristic of Diabetic neuropathy.

Elevated intracellular levels of glucose cause a non-enzymatic covalent bonding with proteins, which alters their structure and inhibits their function. Some of these glycosylated proteins have been implicated in the pathology of diabetic neuropathy.

DIAGNOSIS

Neuropathy is suspected in someone with Diabetes who has pain in a leg or foot. Assess the appearance of the feet, presence of ulceration, and ankle reflexes, the most useful physical examination findings for large fibre neuropathy are an abnormally decreased vibration perception to a 128-Hz tuning fork or pressure sensation with a 5.07 Semmes-Weinstein monofilament. Nerve conduction tests may show reduced functioning of the peripheral nerves, but seldom correlate with the severity of Diabetic peripheral neuropathy and are not appropriate as routine tests for the condition.

HbA1c AND DIABETES

Glycated hemoglobin, HbA1c is a form of hemoglobin that is measured primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose. Normal levels of glucose produce a normal amount of glycated haemoglobin. As the average amount of plasma glucose increases, the fraction of glycated haemoglobin increases in a predictable way. This serves as a marker for average blood glucose levels over the previous 3 months prior to the measurement as this is the half-life of red blood cells.

In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, and retinopathy.²⁵ Monitoring HbA_{1c} in diabetic patients may improve outcomes. When blood glucose levels are high, glucose molecules attach to the hemoglobin in red blood cells. The longer hyperglycemia occurs in blood, the more glucose binds to hemoglobin in the red blood cells and the higher the glycated hemoglobin. Once a hemoglobin molecule is glycated, it remains that way. A buildup of glycated hemoglobin within the red cell, therefore, reflects the average level of glucose to which the cell has been exposed during its life-cycle. Measuring glycated hemoglobin assesses the effectiveness of therapy by monitoring long-term serum glucose regulation. The HbA1c level is proportional to average blood glucose concentration over the previous four weeks to three months.

MEASURING HbA1c

Laboratories use:

1. High-performance liquid chromatography (HPLC): The HbA1c result is calculated as a ratio to total hemoglobin by using a chromatogram.
2. Immunoassay
3. Enzymatic
4. Capillary electrophoresis
5. Boronate affinity chromatography

The approximate mapping between HbA1c values given in DCCT percentage (%) and eAG (estimated average glucose) measurements is given by the following equation:²⁶
$$eAG(\text{mg/dl}) = 28.7 \times A1C - 46.7$$

MAGNESIUM

Magnesium is not a true trace element. It is the fourth most abundant cation in the body and within the cell second only to potassium. The adult human body (70 kg) contains 21 to 28 gm of magnesium (approximately 1 mol). Of this about 60% is in bone, 20% in skeletal muscle, 19% in other cells and 1% in extra cellular fluid.²⁷

BIOCHEMISTRY

An alkaline earth metal, magnesium has chemical properties distinctly different from those of the transition metals. Compared with transition metals magnesium interacts with other chemical species with a stronger electrostatic bonding component and a relative preference for oxygen over nitrogen

atoms. There are two major roles for magnesium in biological systems:

It can compete with calcium for binding sites on proteins and membranes.

It can form chelates with important intracellular anionic ligands, notably adenosine triphosphate (ATP).²⁸

Magnesium catalyses or activates more than 300 enzymes in the body. Magnesium acts as an essential cofactor for enzymes concerned with cell respiration, glycolysis and transmembrane transport of other cations such as calcium and sodium. Notably the activity of Na-K ATPase depends on magnesium. Magnesium can affect enzyme activity by binding the active site of the enzyme (pyruvate kinase, enolase) by ligand binding (ATP-requiring enzymes), by causing conformational changes during the catalytic process (Na-K ATPase) and by promoting aggregation of multi enzyme complexes.

DISTRIBUTION

Magnesium is the fourth most abundant cation in the body and the second most prevalent intracellular cation. The total body magnesium content is approximately 25 g (1.03 mol), of which about 55% resides in the skeleton. One third of skeletal magnesium is exchangeable and probably serves as a reservoir for maintaining a normal extracellular magnesium concentration. About 45% of the body's magnesium is intracellular. The concentration of magnesium in the cells is approximately 1 to 3 mmol/L. In general, higher the metabolic activity of a cell the higher is its magnesium content.

Within the cell magnesium is compartmentalized and most of it is bound to proteins and negatively charged molecules; 80% of cytosolic magnesium is bound to ATP. Significant amounts are found in the nucleus, the mitochondria and endoplasmic reticulum. Free magnesium accounts for 0.5% to 5.0% of total cellular magnesium and it is this fraction that is probably important for enzyme activity. The normal magnesium concentration is approximately 1.6 to 2.4 mg/dl (0.70-0.99). About 55% of magnesium is free, 30% associated with proteins (primarily albumin) and 15% complexed with phosphate, citrate and other anions.²⁹

METABOLISM

Magnesium intakes vary appreciably an approximate range for US and Western European population being 140 to 180 mg/day. The recommended dietary allowance (RDA) for magnesium is 270 – 350 mg/day for adults. The magnesium content of food varies widely. Vegetables containing chlorophyll, seafood, nuts and grains contain appreciable amounts, whereas oils, fats and sugars contain very little. In addition drinking water especially hard water may be major source of magnesium.³⁰

About 20 to 30% of ingested magnesium is absorbed from the gastrointestinal tract in people consuming self-selected diets. However this may vary widely because intestinal absorption is inversely related to magnesium intake. Magnesium absorption is affected by malabsorption syndromes, factors that affect transit time, calcium, phosphate, protein, lactose or ingested alcohol. Vitamin D has not shown to affect magnesium absorption.

The major excretory pathway for absorbed magnesium is through the kidney. The kidneys are the main organs of magnesium homeostasis in maintaining plasma homeostasis in maintaining plasma concentrations. During periods of magnesium depletion kidney magnesium excretion can be markedly reduced. Only 3 to 6 % of the filtered load in the kidney is excreted. Approximately 25% of the filtered

magnesium is reabsorbed in the proximal tubule and 50 to 60% in the ascending limb of loop of Henle. Reabsorption of magnesium in the distal tubule is load dependent. The renal clearance and plasma concentrations are often related to those of those of calcium, phosphate, sodium and potassium. There is evidence for hormonal regulation of the renal clearance of magnesium similar to that of potassium. The major part of magnesium in plasma (about 60-70%) exists as free ions or in the form of various diffusible complexes; the remainder is bound to protein.²⁷

Clinical significance

The best defined manifestation of magnesium deficiency is impairment of neuromuscular junction; examples are hyperirritability, tetany, convulsions and electrocardiographic changes. Magnesium deprivation has been associated with cardiovascular disease through epidemiological evidence that relates low magnesium intake to a high incidence of cardiac deaths, particularly in soft water areas where waterborne magnesium is low and a low incidence of cardiac deaths in hard water areas where magnesium intakes are higher. Hypertension, myocardial infarction, cardiac dysrhythmias, coronary vasospasm and premature atherosclerosis also have been linked to magnesium depletion.³⁰

Human magnesium deficiency as indicated by reduced serum magnesium amounts (hypomagnesemia) occurs with either normal or reduced serum calcium concentrations. Hypomagnesemia may be secondary affect in hypocalcemia or calcium deficient tetany. Yet a hypomagnesemic normocalcemic tetany has been described that can be effectively treated with magnesium supplementation alone. During tetany serum magnesium concentrations of 0.15 to 0.5 mmol/L accompanied by normal serum calcium and pH have been reported. There is evidence that tetany accompanied by hypocalcemia and hypomagnesemia may not be optimally treated with calcium administration alone. Decreased serum potassium concentrations (hypokalemia) have also been found to accompany magnesium depletion. The occurrence of otherwise unexplained hypokalemia or hypocalcemia should suggest magnesium deficiency.

LABORATORY ASSESMENT OF MAGNESIUM NUTRITION

Serum or plasma magnesium concentration

Serum or plasma magnesium concentration provides only an approximate guide to the presence or absence of magnesium deficiency. Hypomagnesemia reliably indicates magnesium deficiency, but its absence does not exclude significant magnesium depletion. The concentration of magnesium in serum has not been shown to correlate with any other tissue pools of magnesium except interstitial fluid.³¹

Magnesium concentration in muscle

Muscle contains approximately 27% of total magnesium. Thus it is an important tissue for magnesium status assessment. Needle biopsy has been used to determine the magnesium concentration in muscle, but this procedure is invasive and requires special skills and the assay is tedious.

Mononucleated white cell magnesium concentration

Mononucleated white cell (MNC) magnesium concentration has been proposed as a possible index of intracellular magnesium. In humans magnesium concentrations in MNCs do not correlate with serum or erythrocyte concentrations but several studies show a correlation between the magnesium concentration of MNC and muscle.³² The magnesium content

of MNCs is reportedly a better indicator of cardiac arrhythmias associated with magnesium deficiency than is the magnesium concentration in serum.

DETERMINATION OF MAGNESIUM

Methods

Serum .magnesium has been measured using a wide variety of techniques including precipitation, titration, fluorometry, photometry, flame emission spectroscopy and AAS.³³ Early methods used ammonium phosphate to quantitatively precipitate magnesium which could then be determined gravimetrically or by analysis of phosphate in the precipitate. The precipitation of magnesium by 8-hydroxyquinolone is the basis for the measurement of magnesium by various techniques. Titrimetric methods have been reported using EDTA with an indicator eriochrome black T in a manner analogous to the titrimetric method described for calcium. Flame emission spectroscopy has been used despite the fact that magnesium is a poor emitter at low temperature and the large quantity of sodium, potassium and phosphate interfere. A number of flourometric methods have been used. Enzymatic methods have now been developed with hexokinase or other enzymes that use Mg-ATP as substrate.³⁴ The rate of this reaction is dependent on the concentration of magnesium in the sample. Coupling hexokinase glucose-6- phosphate-dehydrogenase allows the reaction to be monitored at 340 nm with the formation of NADPH.³⁵ Today photometric methods are more commonly used by clinical laboratories, although AAS considered the reference method is also used by some laboratories.³⁶

Photometric methods

A number of metallochromatic indicators or dyes change colours selectively binding magnesium and have been used to measure it in biological samples. Eriochrome black T, chrome fast blue G and titan yellow are of historical interest they are not widely used today.³⁷

According to the college of American pathologists comprehensive chemistry survey for 1991, calmagite was used by 43% laboratories for magnesium determinations, followed by methylthymol blue and a formazan dye, each with 24% magon at 7% and AAS ay 1%. Calmagite a metallochromatic indicator forms a coloured complex with magnesium in alkaline solution which is measured at 530-550nm.³⁸ A specific calcium chelating agent, EGTA (ethylene glycol-O,O-bis(2-aminoethyl)-N,N-tetra acetic acid, is added to prevent interference by calcium. Potassium cyanide is added to avoid formation of heavy metal complexes. Polyvinylpyrrolidone and surfactants are included to reduce interference from protein and lipemia.³⁹

Methylthymol blue forms a blue complex with magnesium which is measured around 600 nm. EGTA is added to reduce interference by calcium. Magon, or xylidyl blue (1-azo-hydroxy-3-[2,4-dimethylcarboxanilideo]-naphthalene- 1-[2-hydroxybenzene], binds magnesium in alkaline solution causing a spectral shift and forming a red complex.⁴⁰ Calcium and protein interference is eliminated by EGTA and dimethyl sulfoxide respectively.⁴¹

A formazan dye (1,5-bis[3,5-dichloro-2-hydroxyphenyl]-3-formazan carbonitrile) forms a complex with magnesium at alkaline pH which has been measured at 630 nm by thin film reflectance photometry. The thin film reflectance method is unaffected by icteric, lipemic and hemolysed specimens. Elevated calcium levels cause a small but measurable

overestimation.

Atomic absorption spectrometry

Although neutron activation with magnesium is the definitive method for magnesium analysis, clinical laboratories use AAS as the reference method. Magnesium is determined by AAS after diluting the specimen 1:50 with a standard solution of lanthanum hydrochloride to eliminate interference from anions including phosphate and protein and metal oxides. The dilution also reduces viscosity ensuring that absorption rate for aqueous calibrators and specimens are comparable. The specimen is aspirated into an air acetylene flame in which the ground state magnesium ions absorb light from a magnesium hollow lamp (285.2nm). Absorption at 285.2 nm is directly proportional to the ground state magnesium atoms in the flame⁴².

Specimen

Serum is the preferred specimen but heparinised plasma may also be used. Other anticoagulants such as citrate, oxalate and EDTA are not acceptable because they form complexes with magnesium. Magnesium is considered to be stable in serum for days at 4⁰ C and for months when frozen, provided evaporation and lyophilization are avoided. Serum or plasma must be separated from the clot or red blood cells as soon as possible to avoid increased levels due to cell leakage. Because erythrocytes contain higher levels of magnesium than plasma or serum hemolysed samples are unacceptable.

Interference by icterus or lipemia depends on the methods and use of dialysis, bichromate analysis or blanking. Lipemic specimens should be ultracentrifuged. Interference in photometric methods may be overcome with EDTA blanking. Urine specimens should be collected in HCl, 20 to 30 ml of 6 mol/L for 24 hours specimen, to prevent precipitation of magnesium complexes. As with calcium, if acid must be added after collection, the entire specimen must be acidified and heated. Collection of the specimen in acid to prevent precipitation is recommended.

Reference Interval

The reference interval for serum magnesium is approximately 1.6 to 2.4 mg/dL (0.70 - 0.99 mmol/L).²¹ Erythrocytes have magnesium levels approximately three times those of serum.

MAGNESIUM DEFICIENCY:

Causes:

I. PRIMARY NUTRITIONAL DISTURBANCES

- a. Inadequate intake
- b. Total parenteral nutrition
- c. Refeeding syndrome

II. GASTROINTESTINAL DISORDERS

- a. Specific absorptive defects
- b. Malabsorption syndromes
- c. Prolonged diarrhoea
- d. Prolonged nasogastric suction
- e. Pancreatitis

III. ENDOCRINE DISORDERS

- a. Hyperparathyroidism
- b. Hypoparathyroidism
- c. Hyperthyroidism
- d. Primary hyperaldosteronism
- e. Bartter's syndrome
- f. Diabetic Ketoacidosis
- g. Alcoholic Ketoacidosis

IV. CELLULAR UPTAKE OR REDISTRIBUTION

- a. Administration of epinephrine
- b. Acute pancreatitis
- c. Following correction of respiratory acidosis
- d. Massive blood transfusion
- e. Antacid intake

V. CHRONIC ALCOHOLISM, ALCOHOL WITHDRAWAL

VI. INCREASED RENAL EXCRETION

- a. Ethanol ingestion
- b. Idiopathic
- c. Following renal transplantation
- d. Cyclosporine therapy
- e. Aminoglycoside therapy
- f. SIADH

g. Diuretic administration

- Furosemide
- Ethacrynic acid
- Bumetanide
- Acetazolamide
- Thiazides

h. Recovery from acute tubular necrosis

CLINICAL FEATURES

- anorexia, nausea, vomiting and weakness

If Severe,- Paraesthesia, Muscular cramps, Irritability, Decreased attention span, Mental confusion, Fasciculations, Athetoid tetany, Convulsions.

Positive Trousseau and Chvostek signs

Cardiac arrhythmias, disturbances of conduction, ventricular fibrillation and cardiac arrest.

ECG Changes -

- Depression of ST segment
- Flattening of T waves
- QT/QTc prolongation
- Enhanced atrial and ventricular excitability

Hypomagnesemia is a common feature in patients with type 2 diabetes. Although Diabetes can induce hypomagnesemia, magnesium deficiency has also been proposed as a risk factor for type 2 diabetes. Magnesium is a necessary cofactor for several enzymes that play an important role in glucose metabolism. Animal studies have shown that magnesium deficiency has a negative effect on the post-receptor signalling of insulin. On the post-receptor signalling of insulin. Some short-term metabolic studies suggest that magnesium supplementation has a beneficial effect on insulin action and glucose metabolism.¹⁴ There are many other studies and research done previously to find about the relation between magnesium levels and patients of diabetes.

Gillian Grafton, Bunce M, Sheppard MC, Brown G, Baxter MA (1992) demonstrated that magnesium is a positive effector of inositol transport and is capable of promoting a 25 fold increase in the affinity of the transporter for inositol. They suggested that hypomagnesaemia may be linked to the development of diabetic complications via reduction in the rate of inositol transport and subsequent intracellular inositol depletion.⁴³

Garland HO (1992) stated about a potential link between the magnesium deficit of diabetes and several diabetic complication including cardiovascular problems and retinopathy.⁴⁴

Rude RK (1992) suggested that it would be prudent for physicians who treat patients to consider magnesium deficiency as a contributing factor in many diabetic complications and in exacerbation of disease itself. Repletion of the deficiency or prophylactic supplementation with oral

magnesium may help avoid or ameliorate such complications as arrhythmias, hypertension, and sudden cardiac death and may even improve the course of the diabetic condition.⁴⁵

Nadler JL et al (1992) suggested that type 2 diabetic patients have intra cellular Mg²⁺ deficiency and that Mg deficiency may be a key factor in leading enhanced platelet reactivity in type 2 diabetes. Therefore, Mg supplementation may provide a new therapeutic approach to reducing vascular disease in patients with diabetes.⁴⁶

Betrelloni S (1992) showed that the deranged parathyroid hormone-vitamin D axis in IDDM is reversed after normalization of magnesium serum levels by oral magnesium. He also suggested that hypomagnesaemia is involved in the genesis of the altered mineral metabolism in children with IDDM.⁴⁷

Srivastava VK, Chauhan AK, Lahiri VL. (1993) studied the significance of serum magnesium in diabetes mellitus and concluded that all diabetic patients, having normal renal function exhibited hypomagnesemia. They also observed a positive correlation between blood urea level and serum magnesium and it was significant. The magnesium correlated with major diabetic complications too. Thus serum magnesium can be used for prognostic assessment in diabetic individuals.⁴⁸

Resnick LM, Altura BT, Gupta RK, Laragh JH, Alderman MH and Altura BM (1993) suggested that magnesium deficiency, both extracellular and intracellular, is a characteristic of chronic stable mild type 2 diabetes, and as such, may predispose to the excess cardiovascular morbidity of the diabetic state.⁴⁹

White JR, Campbell RK (1993) in their conclusion suggested a link between hypomagnesemia and hyperglycemia, as well as an association between hypomagnesemia and the complications of DM.⁵⁰

Corica F, Allegra A, Di Benedetto A, Giacobbe MS, Romano G, Cucinotta D (1994) evaluated the effects of oral magnesium supplementation on plasma lipid concentrations in patients with NIDDM. They suggested that oral supplementation of magnesium may be useful in the treatment of hyperlipidemia in patients with NIDDM.⁵¹

Isbir T, Tamer L, Taylor A, Isbir M. (1994) stated that the concentrations of copper were higher and the magnesium levels were lower in IDDM patients than in control subjects. They also said these changes may be associated with the development of insulin resistance and it was proposed that patients will improve if trace elements are given as a part of the therapy.⁵²

Ma J, Folsom AK, Melnick SL, et al. (1995) studied the relationships of serum and dietary magnesium (Mg) with prevalent cardiovascular disease (CVD), hypertension, diabetes mellitus, fasting insulin, and average carotid intimal medial wall thickness measured by B mode ultrasound. They concluded that low serum and dietary magnesium may be related to the etiologies of CVD, hypertension, diabetes and atherosclerosis.⁵³

Alzaid AA, Dinnean SF, Moyer TP, Rizza RA. (1995) sought to determine whether insulin-induced stimulation of magnesium uptake is impaired in NIDDM and enhanced by acute hyperglycaemia and concluded that insulin resistance in subjects with NIDDM impairs the ability of insulin to stimulate magnesium as well as glucose uptake.⁵⁴

Nagase N (1996) investigated interrelations between hypertension, ischemic heart disease (IHD) and diabetes mellitus in diabetic subjects and showed that serum Mg level of poorly controlled diabetic patients is lower than that of well

controlled diabetic patients. It also suggested that, magnesium deficient states is one of the cause of insulin resistance.⁵⁵

Corica F, Allegra A, Buemi MJ, et al (1996) their study showed both normotensive and hypertensive diabetics have a reduction in plasma, erythrocyte and platelet concentration of magnesium compared to controls. No significant difference was found between hypertensive and normotensive diabetics with regard to plasma and erythrocyte magnesium.⁵⁶

Tosiello L (1996) stated that low serum magnesium levels has been reported in children with IDDM and through the entire spectrum of adult type I and type II diabetics regardless of the type therapy. Hypomagnesemia has been correlated with both poor diabetic control and insulin resistance in non-diabetic elderly patients.⁵⁷

Corica F, Allegra A, Buemi MJ et al. (1996) evaluated magnesium concentrations in plasma erythrocyte and platelet and plasma and urine levels of the soluble form of intercellular adhesion molecule-1 (SICAM-1) in subjects and concluded that the reduced intraplatelet magnesium content may contribute to the progression on the vascular complications in IDDM subjects with microalbuminuria.⁵⁸

Husmann MJW, Fuchs P, Truttman AC, et al (1997) confirmed findings of reduced circulating ionized magnesium but normal circulating total magnesium in adults with non-insulin dependent diabetes mellitus.⁵⁹

Paolisso G, Barbagallo M (1997) concluded that intracellular magnesium may play a key role on modulating insulin-mediated glucose uptake and vascular tone. They also suggested that a reduced intracellular magnesium concentration might be the missing link helping to explain the epidemiological association between NIDDM and hypertension.⁶⁰

Ewis SA, Abdel Rahman MS (1997) showed a state of low levels of magnesium and glutathione (GSH) in both blood and liver of diabetic animals. Treatment with atenolol alone did not change these levels significantly; however administration metformin or atenolol/metformin increased significantly the GSH levels in both liver and blood, and returned the liver Mg content take to normal values.⁶¹

De Leeuw I, Engelen W, Vertommen J, Nonneman L. (1997) studied the effect of a 10 week intensive oral and IV supplementation of Mg in 11 depleted IDDM patients with stable metabolic control. Ionized Mg decreased and erythrocyte Mg increased significantly together with an increased storage of Mg in the body demonstrated with a classical retention test.⁶²

Jacomella V, Sauser A, Truttman AC, Kuhlmann-Siegenthaler BV, Branchetti MG. (1997) concluded that in healthy humans the circadian pattern of extracellular magnesium is not modulated by the metabolic and hormonal mechanisms that adjust the concentration of glucose.⁶³

De Valk HW, Verkaarik R, Van Rijn HJM, et al (1998) stated that three months oral Mg supplementation of insulin-requiring patients with type 2 DM increased plasma Mg concentration and urinary Mg excretion but had no effect on glycemic control or plasma lipid concentration.⁶⁴

Lima M, Cruz T, Posuda JC, Rodrigues LE, Barbosa K, Cangacu V. (1998) concluded Mg depletion is common in poorly controlled patients with type 2 diabetes, especially in those with neuropathy or coronary disease. More prolonged use of Mg in doses that are higher than usual is needed to establish its routine or selective administration in patients with type 2 diabetes to improve control chronic complications.⁶⁵

Gurlek A, Bayraktar M, Ozaltin N. (1998) suggested that intracellular Mg depletion without significant

hypomagnesemia is related to increased urinary Mg loss in patients with type 1 diabetes. The urinary Mg loss is not correlated with the degree of metabolic control.⁶⁶

De Valk HW (1999) stated that the plasma magnesium level has been shown to be inversely related to insulin sensitivity. Mg supplementation improves insulin sensitivity as well as insulin secretion in type 2 diabetes. Patients with severe retinopathy have a lower plasma magnesium level compared to patients without retinopathy and a prospective study has shown the plasma magnesium level to be inversely related to occurrence or progression of retinopathy.⁶⁷

Mikhail N, Ehsanipoor K (1999) concluded that their data do not support routine Mg supplementation or monitoring in type 2 diabetes.⁶⁸

Kao WH, Aaron R, Folsom H, et al (1999) concluded that low serum Mg level is a strong, independent predictor of incident type 2 diabetes. That low dietary magnesium intake does not confer risk for type 2 diabetes implies that compartmentalization and renal handling of magnesium may be important in the relationship between low serum magnesium levels and the risk for type 2 diabetics.⁶⁹

Corsonello A et al (2000) conducted a study in which they measured fasting plasma glucose, creatinine, creatinine clearance estimate, total cholesterol and triglycerides, and serum ionized magnesium (ion-selective electrodes, ISE) in 30 NIDDM patients with urinary albumin excretion rate (UAER) <20 µg/min (normoalbuminuria), 30 NIDDM patients with microalbuminuria (20 < UAER < 200 µg/min), 30 NIDDM patients with clinical proteinuria (UAER >200 µg/min), and 20 healthy subjects. They found that Serum ionized magnesium levels were significantly reduced in diabetic patients when compared to control subjects (0.39 ± 0.06 vs. 0.58 ± 0.05 mmol/l, $p < 0.001$). Moreover, diabetic patients with micro albuminuria or clinical proteinuria showed a significant decrease in serum ionized magnesium with respect to norm albuminuria group (norm albuminuria: 0.45 ± 0.02 mmol/l; micro albuminuria: 0.36 ± 0.05 mmol/l, $p < 0.001$; clinical proteinuria: 0.35 ± 0.04 mmol/l, $p < 0.001$). Serum ionized magnesium showed a significant negative correlation with plasma HbA1c and triglycerides in both microalbuminuria and clinical proteinuria groups.⁷⁰

Riduara RL, Stamfer MJ, Willet WC, et al. followed 85,060 women and 48,872 men who had no history of diabetes, cardiovascular diseases or cancer at base line for 18 yrs. Magnesium intake was evaluated every 2-4 yrs. Significant inverse relationship between magnesium intake and diabetes risk was found. This study recommends the increased consumption of foods rich in magnesium.¹⁴

Huerta MG, Holmes V F, Roemenich J N, et al. studied 24 obese non diabetic children and 24 sex and puberty matched lean control subjects. Serum magnesium, indices of insulin sensitivity, dietary magnesium intake and body composition were measured. Association between magnesium deficiency and insulin resistance was present during childhood. Serum magnesium deficiency may be secondary to decreased dietary magnesium intake.¹⁵

Martha Rodríguez-Morán, and Fernando Guerrero-Romero. This study was a clinical randomized double-blind placebo-controlled trial. A total of 63 subjects with type 2 diabetes and decreased serum magnesium (serum magnesium levels mmol/l) treated by glibenclamide received either 50 ml MgCl₂ solution (containing 50 g MgCl₂ per 1,000 ml solution) diarrhea, alcoholism, use of diuretic and/or calcium antagonist drugs, and reduced renal function were exclusion criteria.

Homeostasis model assessment for insulin resistance (HOMA-IR) was used as the parameter of insulin sensitivity and glucose and HbA1c as parameters of metabolic control. Oral supplementation with MgCl₂ solution restores serum magnesium levels, improving insulin sensitivity and metabolic control in type 2 diabetic patients with decreased serum magnesium levels.⁷¹

Ishrat K et al (2004) conducted a study involving 100 subjects, 30 diabetics without complication (group I), 40 diabetics with retinopathy (group II) and 30 non diabetic as normal control group (group III). Blood sugar levels, magnesium, cholesterol and triglyceride were analysed from plasma and serum. The results were correlated with degree of diabetic control from the levels of glycosylated hemoglobin. Serum magnesium levels in group II were found to be significantly lowered than in group I. There was also significant difference in magnesium levels of group I and group III. Significant correlation between the glycosylated hemoglobin and magnesium levels was found in this study.⁷²

Yokota K, Kato M, Lister F, et al.(2004) Effects of magnesium (Mg) supplementation on nine mild type 2 diabetic patients with stable glycaemic control were investigated. Water from a salt lake with a high natural Mg content (7.1%) (MAG21) was used for supplementation after dilution with distilled water to 100mg/100mL; 300mL/day was given for 30 days. Fasting serum immunoreactive insulin level decreased significantly, as did HOMA square R (both $p < 0.05$). There was also a marked decrease of the mean triglyceride level after supplementation. The patients with hypertension showed significant reduction of systolic ($p < 0.01$), diastolic ($p = 0.0038$), and mean ($p < 0.01$) blood pressure. The salt lake water supplement, MAG21, exerted clinical benefit as a Mg supplement in patients with mild type 2 diabetes mellitus.⁷³

Srinivasan AR⁷⁴ et al concluded from their study that, Hypertriacylglycerolemia was pronounced in type 2 diabetes mellitus patients with accompanying hypomagnesaemia. Low Mg levels, high TG levels in association with enhanced HbA1c levels could thus serve as a reliable biochemical indicator of insulin status and action without resorting to the usage of criteria for insulin sensitivity and resistance.

Agrawal P et al "Fasting plasma glucose and glycated hemoglobin levels were significantly higher in all the 3 study groups with diabetes as compared to the controls, however, serum magnesium levels were significantly lower ($p < 0.05$). In diabetic patients with coronary atherosclerosis or peripheral vascular disease, a significant negative correlation was observed between serum magnesium and fasting plasma glucose and glycated haemoglobin indicating the role of hypomagnesaemia in diabetic complications⁷⁵.

Arundhati Dasgupta et al documented hypomagnesaemia in 17 (11.33%) patients. Mean HbA1C was 11.9% in the hypomagnesaemic patients compared with 9.8% in controls ($P=0.016$).⁷⁶

Dae Jung Kim, MD et al did the following study and obtained the result as below "During the 20 -year follow-up, 330 incident cases of diabetes were identified. Magnesium intake was inversely associated with incidence of diabetes after adjustment for potential confounders. The multivariable-adjusted hazard ratio of diabetes for participants in the highest quintile of magnesium in take was 0.53 (95 %, 0.32 – 0.86 ; $p < 0.01$) compared with those in the lowest quintile. Consistently magnesium intake was significantly inversely associated with h s-crp, il-6, fibrinogen and HOMA-IR and serum magnesium levels were inversely correlated with

hsCRP and HOMA-IR.⁷⁷

Aradhana Sharma, et al(2007) conducted a cross-sectional study to examine the relationship between serum magnesium in 50 type 1 and type 2 diabetic patients with or without complications and 40 normal healthy persons. Serum magnesium was significantly low in diabetes with complication than without complications ($p < 0.001$). There was strong association between hypomagnesaemia and retinopathy (1.76 ± 0.26), obesity (1.878 ± 0.326) and hypertension (1.75 ± 0.071) and it was statistically significantly ($p < 0.005, 0.042, 0.000$ respectively).⁷⁸

S.S.Antin and Dhananjaya M et al hypomagnesemia is common in type 2 diabetics. Prevalence of hypomagnesemia is significantly higher in diabetics with microvascular complications compared to diabetics with no microvascular complications. Magnesium deficiency is conclusively associated with diabetic retinopathy.⁷⁹

Aims and Objectives

1. To correlate the occurrence of vascular complications with serum magnesium levels in type 2 diabetes mellitus patients.
2. To correlate the level of glycaemic control (HbA1c level) with serum magnesium level.

Materials and Methods

Study Venue

This study was conducted in the Department of Medicine with the help and co-ordination with Department of Biochemistry, PGIMER & Dr. Ram Manohar Lohia Hospital, New Delhi, India.

Study Subjects

The cases for this study were selected from the patients admitted to wards of PGIMER & Dr. Ram Manohar Lohia Hospital, New Delhi, India, and also patients attending medicine OPD.

Inclusion And Exclusion Criteria

INCLUSION CRITERIA

Patients of type 2 diabetes identified by using WHO criteria²³ either with previous vascular complications or those detected during screening.

EXCLUSION CRITERIA

1. Patients with altered kidney function, creatine level $> 1.5\text{mg/dl}$ and established chronic kidney disease.
2. Patients on drugs affecting magnesium levels.
3. Chronic alcoholic.
4. Patients with mal absorption and chronic diarrhoea.
5. Patients with chronic respiratory disease.

STUDY DESIGN

100 Patients, inclusive of in-patients and outpatients attending Dr R.M.L Hospital who were suffering from diabetes and vascular complications (either present previously or detected by screening) were selected randomly and their serum levels of magnesium were measured by a cross sectional observational study.

Methods

All subjects underwent detailed history, complete physical examination, examination of previous medical records and

relevant laboratory investigations with special reference to diabetes mellitus.

History consisted of information on treatment of diabetes (duration, drugs and compliance), smoking (duration and amount), history suggestive of vascular complications caused by diabetes mellitus. Physical examination was performed to detect various vascular complications of diabetes mellitus. The physical examination also included examination of cardiovascular, respiratory, abdominal and central nervous system (proforma given as annexure).

Diagnosis of Diabetes Mellitus

Patients with diabetes mellitus were those patients having symptoms of polyuria, polydipsia and polyphagia and family history of diabetes. As all the subjects in the study were previously diagnosed Diabetic fasting sugars and post prandial sugars were recorded. The glycosylated haemoglobin levels were measured with HbA1c kit using blood collected in EDTA vials.

Diagnosis of Vascular Complications

Patients with diabetes mellitus were screened for vascular complications both micro and macro vascular complications. Microvascular complications included nephropathy, neuropathy and retinopathy, while macro vascular complications include stroke, coronary events and peripheral vessel disease. The patients either had pre-existing vascular complication or these complications were not apparent at the time of inclusion. The latter group patients were individually screened for the presence of vascular complications associated with diabetes mellitus. The emphasis was on just presence or absence of vascular complications, not on the duration of complications. The duration of diabetes, and the distribution of complications among the study group were also recorded. The serum magnesium levels were measured in all the patients.

NCCT – HEAD

Non contrast computerised tomography was done using PHILIPS, BRILLANCE (Germany). It was used in patients who give history suggestive of stroke to rule out cerebro vascular events. The patients with stroke were identified by signs like hyperdense segment of a vessel, representing direct visualisation of the intravascular thrombus or embolus. Other signs included loss of grey-white matter differentiation, and hypoattenuation of deep nuclei lentiform nucleus, cortical hypodensity with associated parenchymal swelling with resultant gyral effacement.

ECHOCARDIOGRAPHY

Echocardiography was done using PHILIPS, HD11XE (Italy). It was used in patients to detect coronary artery disease in patients who give history of chest pain and breathlessness. The patients with myocardial infarction was identified by the fact that, in a (in)complete occlusion of coronary artery by intraluminal thrombus, downstream blood flow is reduced resulting in impaired contractility of affected segments, i.e. wall motion abnormalities.

DOPPLER

The Doppler of lower limbs were done in patients who give history of non-healing ulcers, using PHILIPS, SARONNO (Italy) to detect peripheral vessel disease. The patients with artery disease had decreased flow in blood vessels indicated by blue colour in Doppler along with atheromatous plaque.

MICROALBUMINURIA

Urine for micro albuminuria was done in patients to rule out nephropathy. The patient's serum creatinine was measured. Those with creatinine more than 1.5 mg/dl were excluded from the study.

OPHTHALMOSCOPY

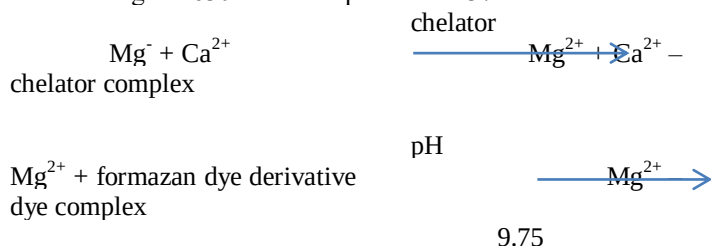
Fundus examination with help of ophthalmoscope was done in patient after dilation of pupils to rule out retinopathy. The patients with retinopathy had blood vessel leakage, exudates, macular edema and nerve damage.

MONO FILAMENT TEST

Monofilament test was done in patients who gave history of tingling and numbness to rule out neuropathy. Pressure sense was tested using 5.07 Semmes-Weinstein monofilament. The 10 pressure points of soles were tested; the patients who were unable to detect more than 3 points were diagnosed to have neuropathy.

MEASUREMENT OF SERUM MAGNESIUM

The serum magnesium levels were measured by using VITROS Mg Slide method which was performed using the Vitros Mg slides and the Vitros chemistry products Calibrator Kit 1 on Vitros chemistry system. Vitros Mg slide is a multi-layered, analytical element coated on a polyester support. A drop of patient sample was deposited on the slide and was evenly distributed by the spreading layer to the underlying layer, magnesium (both free and protein bound) from the sample then reacts with Formazan dye derivative in the reagent layer. The high magnesium affinity of the dye dissociates magnesium from binding protein. The resulting magnesium – dye complex causes shift in the dye absorption max. The amount of dye complex formed is proportional to magnesium concentration present in the sample and is measured by reflection density. The calorimetric absorption is at wave length λ 630 nm at temperature of 37 °C.



OTHER LABORATORY INVESTIGATIONS

- Hemogram, total leucocyte count, differential leucocyte count, platelet count
- Kidney function tests – serum urea, serum creatinine
- Serum electrolytes – sodium and potassium levels
- Body mass index of the subject.

STATISTICAL ANALYSIS

All data was recorded in a prospective database (excel). The observed data for the various parameters was presented in terms of minimum, maximum, normal, abnormal and mean $\pm$ standard deviation (S.D.) for a cross sectional analysis. Further analysis of categorical data was done using the Chi – square test.

The level of statistical significance was taken as $p \leq 0.05$. The data was analysed using the SPSS statistical software, version 20.0.

Observation and Results

The study “Relation between serum Magnesium levels and vascular complications in patients of type 2 Diabetes Mellitus”, is a cross sectional observational study with the objective to correlate the serum levels of Magnesium in patients of type 2 Diabetes Mellitus with vascular complications. All the vascular complications both micro and macro-vascular complications were taken and studied. The study was conducted in Department of Internal Medicine with help of Department of Bio-chemistry, PGIMER, DR R.M.L Hospital, New Delhi.

The study included 100 patients with Type 2 Diabetes from OPD and inpatient services at Dr. Ram Manohar Lohia Hospital, New Delhi.

TABLE – 1 (Figure -1):- Showing the age distribution of subjects included in the present study.

Age Interval (Years)	Number Of Patients	Percentage
40 – 49	24	24%
50 – 59	41	41%
60 – 69	35	35%

The patients included in the study were grouped into three classes of 10 year interval. 41% (41) of patients were in age group of 50 to 59 years, 35% (35) of patients were in age group 60 to 69 and 24% (24) of patients were in age group 40 to 49 years.

FIGURE 1

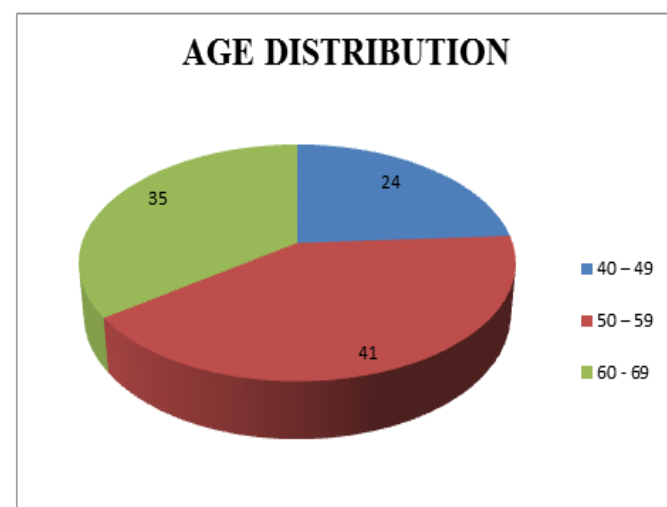


TABLE – 2 (Figure 2):- Showing the distribution of sex among the study population included in the study.

Sex	Number Of Patients	Percentage
MALE	53	53%
FEMALE	47	43%

Of all the diabetics included in the study males were 53 (53%) and total females were 43 (43%).

FIGURE 2

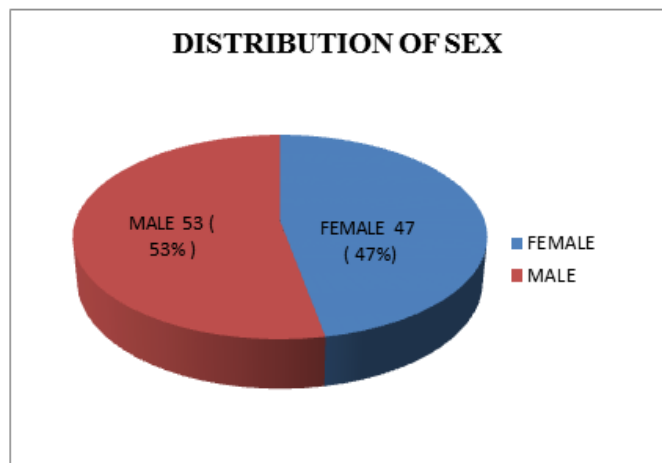


TABLE – 3: Showing complications present among the study population.

Complications	Total Number Of Patients
Coronary Disease	15
Stroke	18
Peripheral Vessel Disease	10
Retinopathy	21
Neuropathy	21
Nephropathy	58

Among the patients screened for vascular complications, 15 subjects suffered from coronary artery disease identified by abnormal Echocardiography, 18 patients had stroke evidenced by abnormal CT head, 10 patients showed abnormal lower limb Doppler studies had peripheral vessel disease, 21 patients had retinopathy identified by ophthalmoscopy, 21 patients had peripheral neuropathy identified by using Monofilament test and almost 58 patients (more than half of the subjects) suffered from nephropathy detected by using urine for micro albuminuria.

TABLE – 4;- Showing the duration of diabetes in years among the study subjects in relation to individual vascular complications.

Duration Of Diabetes (In Years)	0 TO 5	5 TO 10	10 TO 15
No Of Patients	52	29	19
Coronary Artery Disease	8	4	3
Stroke	12	4	2
Peripheral Vessel Disease	4	4	2
Retinopathy	8	6	7
Nephropathy	29	16	13
Neuropathy	9	7	5

The subjects included in the study were grouped into three depending on the duration of diabetes viz 0 to 5 years, 5 to 10 years and 10 to 15 years, with 52 patients (52%) in first group, 29 patients (29%) in second group and 19 patients (19%) in third group, respectively. In group 1 (0 to 5 years) 8 patients had coronary artery disease, 12 patients had stroke, 4 patients had peripheral vessel disease, 8 patients had retinopathy, 29 patients had nephropathy and 9 patients had neuropathy respectively. In group 2 (5 to 10 years) 4 patients had coronary

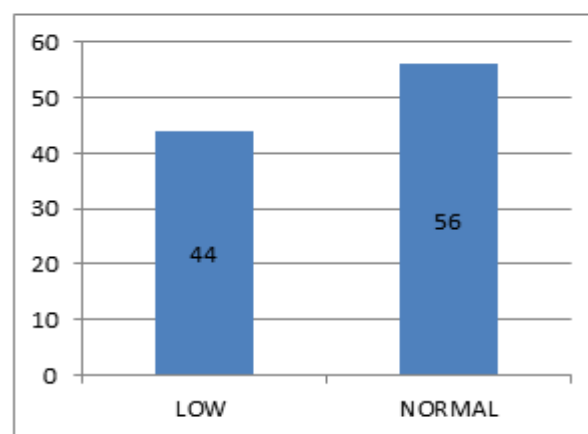
artery disease, 4 patients had stroke, 4 patients had peripheral vessel disease, 6 patients had retinopathy, 16 patients had nephropathy and 7 patients had neuropathy respectively. In group 3 (10 to 15 years) 3 patients had coronary artery disease, 2 patients had stroke, 2 patients had peripheral vessel disease, 7 patients had retinopathy, 13 patients had nephropathy and 5 patients had neuropathy, respectively.

TABLE – 5 (Figure 3);- Showing the serum magnesium levels among the study population.

Magnesium Levels	Number Of Patients	Percentage
NORMAL LEVELS (1.6 to 2.4 mg/dl)	56	56%
LOW LEVELS (< 1.5 mg/dl)	44	44%

Among 100 patients of diabetes with vascular complications, 44 patients (44%) had serum levels of magnesium levels less than 1.5 mg/dl. The remaining 56 patients (56%) had normal serum magnesium levels.

FIGURE 3



Low – serum magnesium levels ≤ 1.5 mg/dl

Normal – serum magnesium levels 2.5 to 1.6 mg/dl

TABLE – 6;- Shows the distribution of serum magnesium levels in various complications of type 2 diabetes.

SERUM MAGNESIUM	NORMAL (1.6 TO 2.5 mg/dl)	LOW (≤ 1.5 mg/dl)
Coronary Artery Disease	7	8
Stroke	8	10
Peripheral Vessel Disease	5	5
Retinopathy	10	11
Nephropathy	32	26
Neuropathy	11	10

Among the normomagnesimics, 7 patients had coronary artery disease, 8 patients had stroke, 5 patients had peripheral vessel disease, 10 patients had retinopathy, 32 patients had nephropathy and 11 patients had neuropathy. Among the hypomagnesimics, 8 patients had coronary artery disease, 10 patients had stroke, 5 patients had peripheral vessel disease, 11 patients had retinopathy, 26 patients had nephropathy and 11 patients had neuropathy.

TABLE - 7 (Figure 4);- Showing distribution of serum magnesium in patients with coronary artery disease(CAD).

Serum Magnesium	NO CAD	CAD
Hypomagnesim	36	8
Normomagnesim	49	7

Among normomagnesimics 7 people suffered from coronary artery disease and 8 of the hypomagnesimics suffered from coronary disease. The results obtained were not statistically significant.(Pearson chi square test with 95% confidence interval and degree of freedom 1 showed P value of 0.43)

FIGURE 4

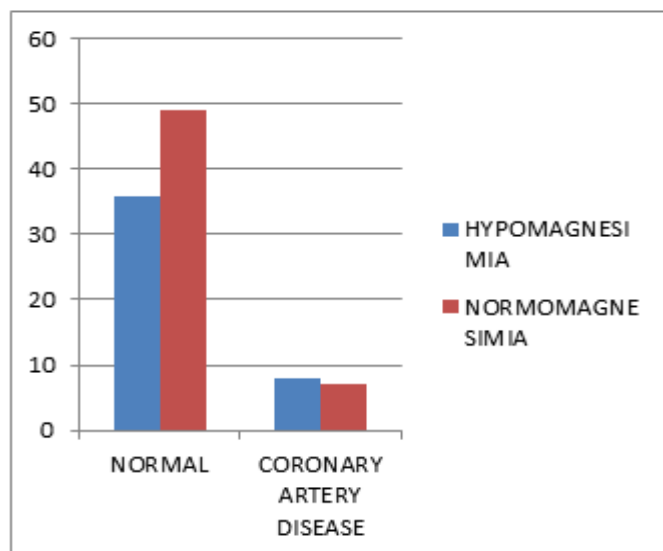


TABLE - 8 (Figure 5);- Showing distribution of serum magnesium in patients with stroke.

Serum Magnesium	No Stroke	Stroke
Hypomagnesim	34	10
Normomagnesim	48	8

Among hypomagnesimics 10 patients had stroke and 8 normomagnesimics had stroke though occurrence of stroke is high in hypomagnesimics, the result is not statistically significant. (Pearson chi square test with 95% confidence interval and degree of freedom 1 showed P value of 0.275)

FIGURE 5

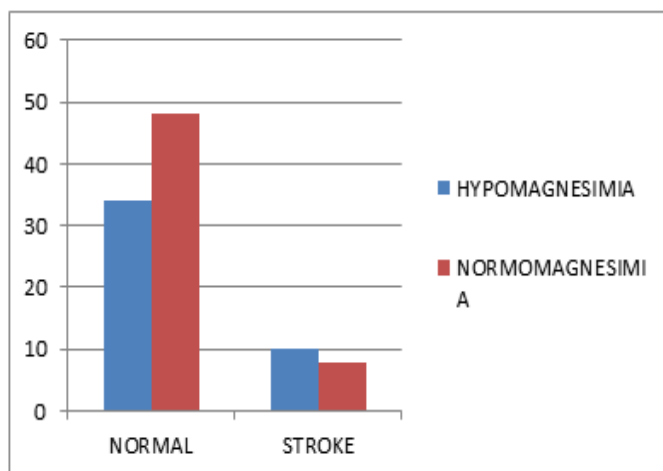


TABLE - 9 (Figure 6);- Showing distribution of serum magnesium in patients with peripheral vessel disease (PVD).

Serum Magnesium	NO PVD	PVD
Hypomagnesim	39	5
Normomagnesim	51	5

Among hypomagnesimics 5 had peripheral vessel disease and among normomagnesimics 5 had peripheral vessel disease. Though occurrence of peripheral vessel disease is more in patients of hypomagnesim the result is not statistically significant. (Pearson chi square test with 95% confidence interval and degree of freedom 1 showed P value of 0.687)

FIGURE 6

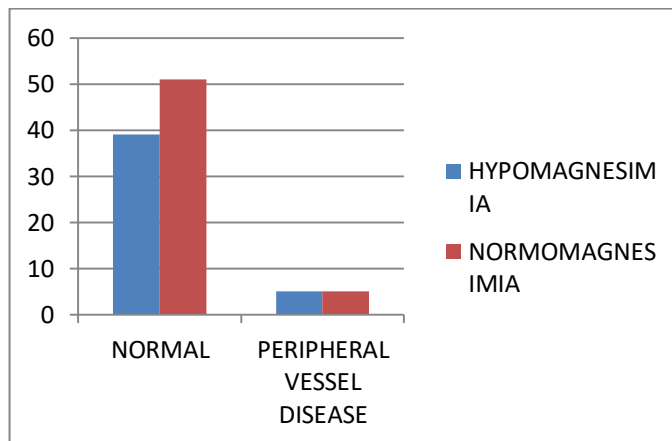


TABLE - 10 (Figure 7);- Showing distribution of serum magnesium in patients with retinopathy.

Serum Magnesium	No Retinopathy	Retinopathy
Hypomagnesim	33	11
Normomagnesim	46	10

Among hypomagnesimics 11 had retinopathy and among normomagnesimics 10 had retinopathy. Though the occurrence of retinopathy is more in hypomagnesimics, the result is not statistically significant. (Pearson chi square test with 95% confidence interval and degree of freedom 1 showed P value of 0.275)

FIGURE 7

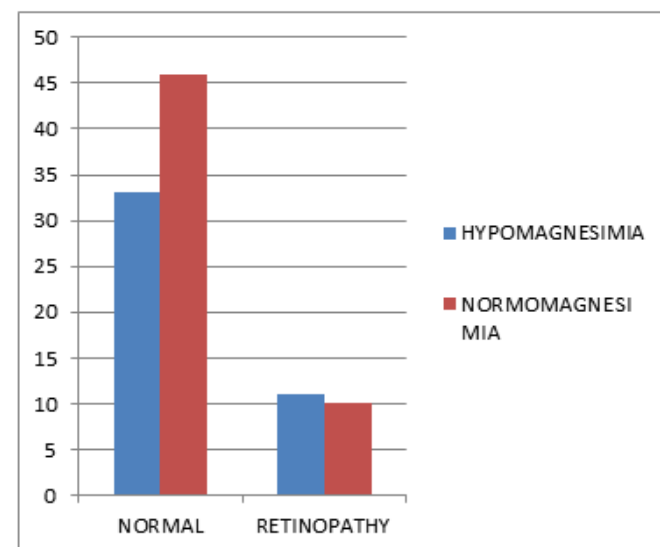


TABLE - 11 (Figure 8);- Showing distribution of serum magnesium levels in patients with neuropathy.

Serum Magnesium	No Neuropathy	Neuropathy
Hypomagnesimia	34	10
Normomagnesimia	45	11

Out of the 21 patients who had neuropathy, 10 (29.4%) were hypomagnesimics and 11 (24.4%) were normomagnesimics. Though, occurrence of neuropathy was more in hypomagnesimics the results were not statistically significant. (Pearson chi square test with 95% confidence interval and degree of freedom 1 showed P value of 0.707)

FIGURE 8

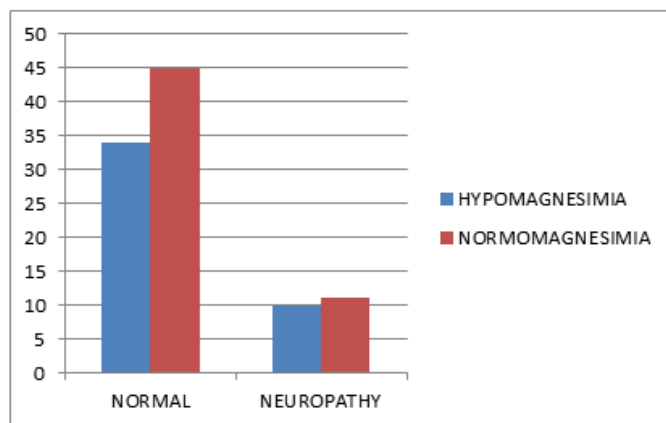


TABLE – 12 (Figure 9):- Shows distribution of magnesium levels among patients with nephropathy.

SERUM MAGNESIUM	NO NEPHROPATHY	NEPHROPATHY
HYPOMAGNESIMIA	18	26
NORMOMAGNESIMIA	24	32

Of the 58 patients of nephropathy, 26 (59.1%) were hypomagnesimics and 32 (57.1%) were normomagnesimics. Though the occurrence of nephropathy is slightly more in patients of hypomagnesimia it was not significant statistically. (Pearson chi square test with 95% confidence interval and degree of freedom 1 showed P value of 0.505)

FIGURE 9

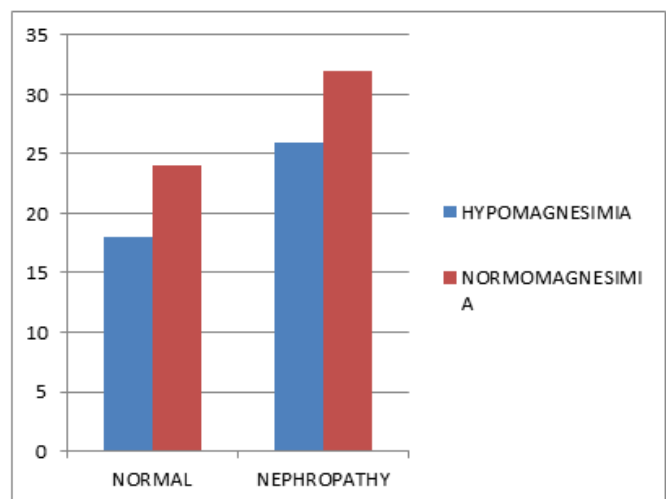


TABLE - 13;- Showing distribution of serum magnesium in

patients with poor and good glycemic control (HbA1c < 7%) .

Glycemic Control	Number Of Patients	Patients With Hypomagnesimia	Patients With Normomagnesimia
GOOD (HbA1c < 7%)	18	5	13
POOR (HbA1c ≥ 7%)	82	39	43

Among the 18 patients with good glycaemic control (HbA1c < 7%) 5 (27.7%) patients had hypomagnesimia and among the 82 patients with poor glycaemic control 39 (47.5%) patients had serum magnesium levels less than 1.5 mg/dL. Though hypomagnesimia is more common in patients of poor glycemic control, the result was not statistically significant. (Pearson chi square test with 95% confidence interval and degree of freedom 1 showed P value of 0.126)

Discussion

Diabetes mellitus incidence is on the rise. As the life style and culture of people has changed the patient with diabetes presenting to hospital with vascular complications are also on rise. There are many studies correlating the role of trace elements in vascular complications of diabetes. In present study we study the level of serum magnesium among diabetic patients with vascular complications. Magnesium is mainly an intracellular cation, with less than 2% of total body content present in the extracellular fluids⁸⁰. 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 status⁸¹.

Magnesium is a cofactor in both glucose transporting mechanisms of cell membrane and various enzymes important in carbohydrate oxidation. Magnesium deficiency has been reported in the previous studies in patients with type 2 diabetes. However, some workers have also reported normal and even high levels⁸².

The present study included 100 type 2 diabetic patients; they were diagnosed of previous vascular complications present already or screened for vascular complications by appropriate test. Serum magnesium levels were determined in all these subjects. The age of all the subjects included in the study ranged from 40 to 67 years with mean of 55.85 years. The study done by Srivastava V K et al the mean age of study population was 52.65 years. The mean BMI of the subjects in the present study was 25.34 with range of BMI from 40.01 and 19.05. In the study done by, De Valk HW⁶¹ the mean BMI of the subjects was 25.68 which was very similar to present study.

In the present study, the presence of complications and the duration of the illness were compared. No correlation was obtained among the duration of diabetes and presence of vascular complications. The usual dictum of retinopathy preceding nephropathy, as suggested by El Asrar et al⁸⁴ was not obtained as in the study 58 patients had nephropathy, whereas only 21 patients had neuropathy. Only 10 patients had both retinopathy and nephropathy. This might be because of false negative results due to hand held ophthalmoscope, moreover the gold standard test for retinopathy is fluorescein angiography which was not done in our subjects.

In the present study, the presence of vascular complications among the patients with normal magnesium levels and patients with low magnesium levels were analysed for each individual vascular complications. It was not found to be statistically significant. The study done by Ma J et al showed association of hypomagnesemia with retinopathy no such correlation was obtained in present study. This might be because in the present study the detection of patients with retinopathy was low because of use of hand held ophthalmoscope.

The present study analysed the association of vascular complications with serum magnesium levels. It was found that there is no significant association between low serum magnesium levels and occurrence of individual vascular complications. The study done by Mikhail N et al showed significant hypomagnesemia in patients of poorly controlled diabetics. This might be because in study done by Mikhail N et al the mean HbA1c of the subjects was 9.86%, but in the present study the mean HbA1c was 8.28%.

A very similar cross sectional observational study which included 100 patients with type 2 diabetes (69 men, 31 women) was done by Mohan et al. The present study also included 100 patients with type 2 diabetes (53 men and 47 women). In the study done by Mohan K et al, the subjects were grouped with regards to their age, duration of diabetes, mode of diabetic treatment, glycemic control, presence/absence of comorbidities (ischemic heart disease and hypertension) and presence/absence of diabetic complications (diabetic retinopathy, diabetic neuropathy, diabetic nephropathy). The study done by Mohan K et al and the present study is very similar.

TABLE – 14;- Shows the comparison between study done by Mohan K et al and the present study.

Charecteristics	Mohan K et al	Present Study
No. Of Subjects	100	100
AGE (In Years)	58.67 (40 - 76)	55.85(40 – 67)
Men	69	53
Women	31	47
Co Morbidites		
Ischemic Heart Diseae	15	15
Diabetic Retinopathy	38	21
Diabetic Neuropathy	15	21
Diabetic Nephropathy	19	58
Hba1c > 7%	67	82

This present study did not include people with hypertensive disease. The relation between HbA1c and serum magnesium levels were not significant in study done by Mohan K et al, the present study also did not show any relationship though the present study had higher patients with poor glycaemic control (mean HbA1c – 8.287, standard deviation 1.6245)(Chi-square value, P=0.088 P value is not less than 0.05, not statistically significant when at 95% confidence interval and degree of freedom of 1). The duration of diabetes did not significantly predict serum magnesium concentration.

The study done by Mohan K et al had prevalence of 35% hypomagnesemia, in the present study, 44% of subjects had hypomagnesemia.

In a study Alzaida et al¹⁸⁵ have found that cellular uptake of magnesium is normally stimulated by insulin. So insulin treatment may enhance cellular magnesium uptake and result

in increased prevalence of hypomagnesemia. Since in present study, the emphasis on insulin treatment was not made, so no significant relation was obtained.

Nagase N (1996) studied the interrelationships between hypertension, ischemic heart disease and diabetes mellitus and diabetes mellitus in the diabetic subjects without ischemic heart disease or with ischemic heart disease and subjects with ischemic heart disease which were not complicated with diabetes mellitus. Their results showed serum magnesium levels of diabetes mellitus (1.90 ± 0.37) was significantly lower than that of normal controls (2.30 ± 0.32). They also concluded that serum magnesium level of poorly controlled diabetic patients is lower than that of well controlled diabetic patients. These results suggested that magnesium deficient state is one of the causes of insulin resistance. The present study did not evaluate the interrelations between hypertension and ischemic heart disease..

Since there was follow up as a part of present study. Hence change in magnesium states with respect to improvement or worsening of diabetic state in the long run was not studied. This study focuses on estimating magnesium levels in type 2 diabetics at a given point (during admission) but not on therapeutically correcting hypomagnesaemia or otherwise (not correcting) in the future course of the disease and its outcome.

Conclusion

The study of serum magnesium levels in patients of type 2 diabetes mellitus with vascular complications showed that 44% of them have hypomagnesemia, indicating the significance of role of magnesium in endothelial function and vascular complications.

- The prevalence of hypomagnesemia with each individual vascular complication is high but not statistically significant.
- The prevalence of hypomagnesemia in poorly controlled diabetics HbA1c > 7% is high but results are not statistically significant.
- Hence it is worthwhile estimating magnesium levels in type 2 diabetes mellitus patients and probably correlates their relationship with various complications.
- Magnesium can be considered as nutritional supplement in patients of diabetes to reduce the incidence of vascular complications.

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