HYPOMAGNESEMIA IN DIABETES MELLITUS- PREVALENCE, CAUSES AND CORRELATION WITH THE COMPLICATIONS AND COMORBIDITIES OF DIABETES MELLITUS

A Dissertation submitted in part fulfillment of M.D. Branch-1

(General Medicine) examination of the Tamilnadu

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March 2009.

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CERTIFICATE

This is to certify that the dissertation entitled ***"Hypomagnesemia in diabetes mellitus- Prevalence, causes and correlation with the complications and co-morbidities of Diabetes mellitus"*** is the bonafide original work of Dr. Justy Antony towards the M.D. Branch-1 (General Medicine) Degree Examination of the Tamil Nadu Dr. M.G.R University, Chennai to be conducted in 2009.

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C E R T I F I C A T E

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INTRODUCTION

Diabetes mellitus is a very common endocrinopathy, and occurs as a result of absolute or relative deficiency in insulin. It is one of the main threats to human health in the 21st century, being a leading cause of death. Several distinct types of DM exist and are caused by a complex interaction of genetic and environmental factors (3). Diabetes is associated with various microvascular, macrovascular, and metabolic complications (2).

Hypomagnesemia has been proposed to be correlated to diabetes, its pathogenesis, complications and comorbidities. Studies have shown that the prevalence of hypomagnesemia among diabetics range from 13.5 to 47.7% (4). Magnesium being the fourth most abundant cation in the body plays an important role in over 300 enzymatic reactions. Thus magnesium deficiency has been proposed as a possible contributor to diabetic complications (2). Of note is the large body of evidence that shows a link between hypomagnesemia and reduction of tyrosine-kinase activity at the insulin receptor level, which may result in the impairment of insulin action and development of insulin resistance (5, 6). There is also evidence that magnesium supplementation may be associated with reduction in the incidence of diabetes and diabetic complications and co-morbidities (7-9).

Although there are several studies in the literature both from the west and from India on the role of magnesium in diabetic complications, several aspects of association between the two are unclear (2).

This study was undertaken to assess the prevalence of hypomagnesemia in our diabetic population, and to propose probable predictors for the same.

AIM

To determine the prevalence and predictors of hypomagnesemia in the outpatient diabetic population at Christian Medical College Hospital, and to describe the association with diabetic complications and co-morbidities.

OBJECTIVES

1. To determine the prevalence of hypomagnesemia in type 2 diabetes mellitus.
2. To ascertain if there was a correlation between hypomagnesemia and the presence of microvascular and macrovascular complications of type 2 diabetes mellitus.
3. To correlate hypomagnesemia with comorbidities like hypertension and dyslipidemia.
4. To evaluate the possible mechanisms of hypomagnesemia in diabetes in particular urinary magnesium excretion, drug history, dietary and drinking water magnesium content.

LITERATURE REVIEW

Introduction

Diabetes mellitus is a group of metabolic disorders that share the phenotype of hyperglycemia. It results from defects in insulin secretion, insulin action or both (3, 10).

Diabetes is one of the main threats to human health in the 21st century. Several epidemiological studies confirm that diabetes is one of the most common non-communicable diseases globally, and is the fourth or fifth leading cause of death in most developed countries.

Diabetes can affect nearly every organ system in the body. It can cause blindness, lead to end stage renal disease, lower-extremity amputations and increase the risk for stroke, ischaemic heart disease, peripheral vascular disease, and neuropathy.

Classification of Diabetes Mellitus

The etiological classification of diabetes mellitus is as follows (Table:1) (3):-

Types	**Etiopathogenesis**
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 to predominantly an insulin secretory defect
III. Other specific types	a) Genetic defects in Beta Cell Function/Insulin secretion b) Genetic defects in Insulin Action c) Diseases of the Exocrine Pancreas d) Endocrinopathies e) Drug or Chemical Induced f) Infections g) Uncommon Immune forms h) Genetic syndromes with diabetes
IV. Gestational diabetes mellitus	Insulin resistance related to the metabolic changes of late pregnancy

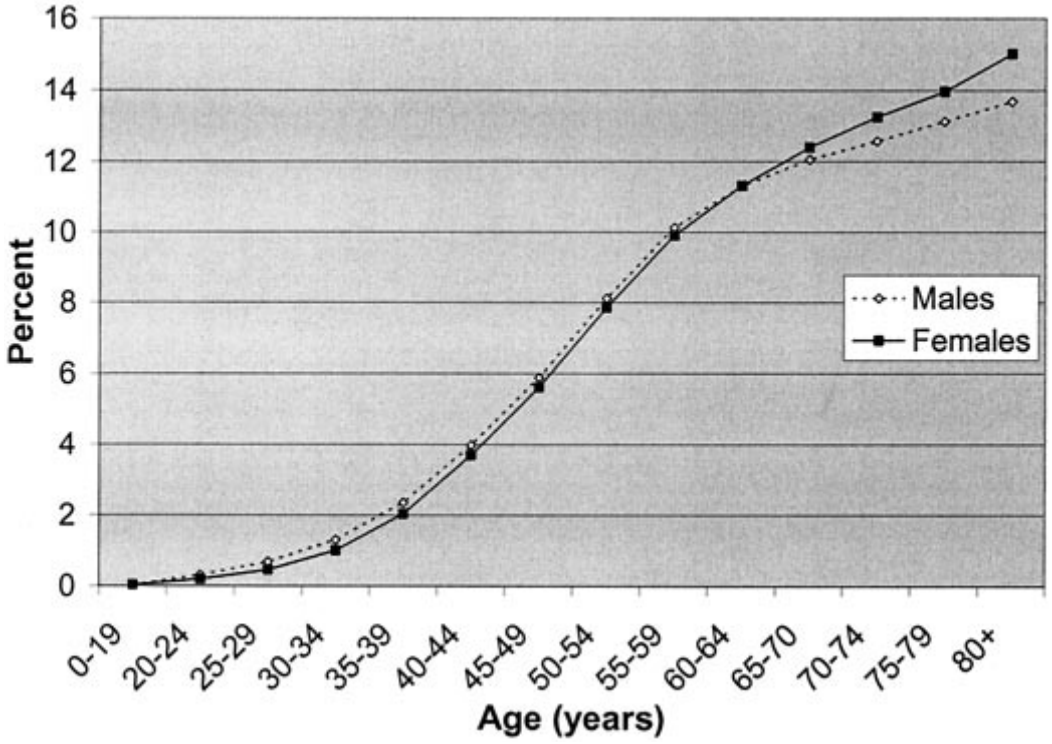
Epidemiology of Diabetes

The prevalence of diabetes ranges from nearly 0 per cent in New Guinea to 50 per cent among Pima Indians(11). The past two decades have seen an explosive increase in the number of people diagnosed with diabetes world-wide.

World data: - The World Health Organization (WHO) estimated that there were 135 million diabetics in 1995 and that this number would increase to 300 million by the year 2025. India is the first among the 10 countries where the prevalence is estimated to increase from 31.7 million in 2000 to 79.4 million by 2030 (12).

Global diabetes prevalence by age and sex for 2000 (12). Borrowed from the Global prevalence of Diabetes.

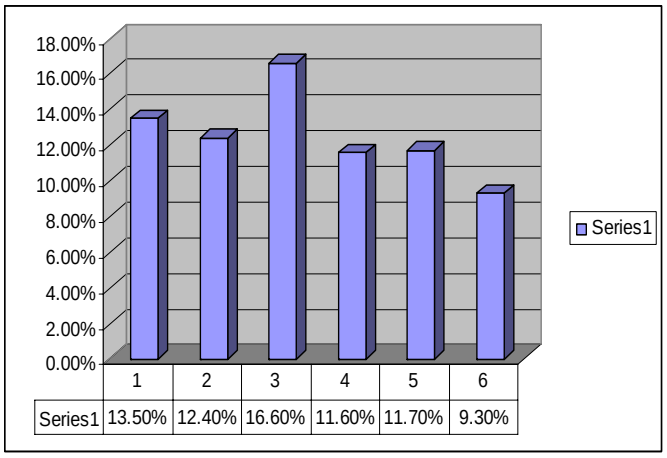
Figure 1: Estimates for the year 2000 and projections for 2030



Indian data: - India leads the world today with the largest number of diabetics in any given country. In the 1970s, the prevalence of diabetes among urban Indians was reported to be 2.1 per cent and this has now risen to 12.1 per cent (13). Moreover, there is an equally large pool of individuals with impaired glucose tolerance (IGT), many of whom will develop type 2 diabetes mellitus in the future (13).

South Indian data: **-** The prevalence in the southern part of India is higher compared to other parts of India, as shown in the (graph 1) below: -

Graph 1- Prevalence of diabetes in various parts of India



	1	2	3	4	5	6
Series1	13.50%	12.40%	16.60%	11.60%	11.70%	9.30%

South India
1. Chennai- 13.5%
2. Bangalore- 12.4%
3. Hyderabad- 16.6%

Northern India
4. New Delhi- 11.6%

Eastern India
5. Kolkatta- 11.7%

Western India
6. Mumbai- 9.3%

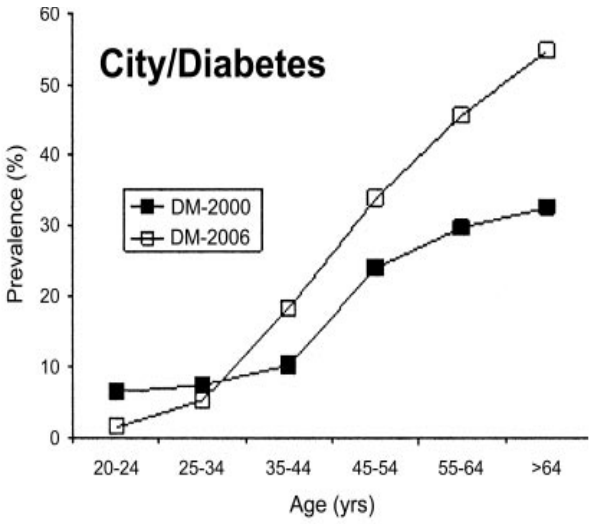
The study also suggested that there was a large pool of subjects with IGT, 14% with a high risk of conversion to diabetes (NUDS study)(14).

Tamil Nadu data: - In a recent study published in 2008, it was found that the prevalence of diabetes in Chennai city was 18.6 %[16.6 –20.5]), compared to a nearby village, which had a prevalence of 9.2% [95% CI 8.0 –10.5], *P*< 0.0001 (1).

Graph 2. The difference in the diabetes prevalence in Chennai city in the years 2000 and 2006.

This graph shows the difference in the diabetes prevalence in Chennai city in the years 2000 and 2006.
The probable risk factors proposed were:-
a) Increased urbanization
b) Higher education
c) Family history
d) Age
e) BMI/ waist circumference
f) City > town > village life

This graph borrowed from- Ambady Ramachandran etal. Diabetes Care, 2008. 31: p. 893-898. (1)



Diagnosis of Diabetes Mellitus

According to American Diabetes Association 2007, a diagnosis of Diabetes mellitus can be made, if the blood sugar values fulfill any one of the following criteria(10):-

Criteria for the diagnosis of diabetes
1. Symptoms of diabetes and a casual plasma glucose >/=200 mg/dl (11.1 mmol/l). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.
OR
2. FPG >/=126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.
OR
3. 2-h plasma glucose >/=200 mg/dl (11.1 mmol/l) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75-g anhydrous glucose dissolved in water.

Diagnosis of Impaired Fasting glucose	**Diagnosis of Impaired glucose tolerance**
Fasting glucose >100mg/dl and <126mg/dl	*2 hours postprandial glucose >140mg/dl and <200mg/dl.*

The 75 gm OGTT is more sensitive, more specific than fasting plasma glucose in diabetes but is poorly reproducible and rarely performed in practice.

Complications of Diabetes Mellitus

Diabetes mellitus causes both microvascular and macrovascular complications, as listed in Table 2. The microvascular complications include- neuropathy, nephropathy and retinopathy, whilst the macrovascular complications are ischemic heart disease, cerebrovascular disease and peripheral vascular disease(3).

Table 2. Complications of diabetes mellitus:

Microvascular	Macrovascular	Others
•Retinopathy (nonproliferative or proliferative) •Neuropathy •Nephropathy	•Cardiovascular disease •Peripheral vascular disease •Cerebrovascular disease	**GI** • Gastroparesis • Diarrhea **Genitourinary** **Lower extremity** • Amputation •Foot ulcers and infections

I. Neuropathy

It is the commonest symptomatic complication of diabetes. It can be seen in 15-40% of the diabetic population from various studies. It can be classified as (15):

- Subclinical neuropathy
- Diffuse clinical neuropathy
- Focal neuropathy
- Mononeuropathy

- Entrapment syndromes
- Autonomic neuropathy

Recognised methods (15):

a) 10 g Semmes- Weinstein Monofilament for assessment of sense of touch

b) 128 Hz tuning fork or biothesiometer- for testing vibration

Impaired vibration sense is an early sign of neuropathy, hence its assessment is an important element of the neurological examination of the patient with diabetes.

II. **Nephropathy**

Mogensen has classified diabetic nephropathy into several stages(15):

1. Stage of glomerular hyper filtration and renal enlargement
2. Early glomerular lesions or silent stage with normal albumin excretion
3. Incipient nephropathy or the microalbuminuric stage
4. Clinical or overt diabetic nephropathy: proteinuria and declining GFR
5. End stage renal disease

Microalbuminuria

Microalbuminuria is defined as 30- 299 microgms of albumin/ mg of creatinine in a spot urine sample or 30-500 mg/day in a 24 hour urine collection (10). It can also occur with uncontrolled hypertension, fever, strenuous exercise, poor glycemic control or CCF. It may be seen 5 to 10 years after the onset of diabetes in 40% of type 1 diabetic patients. In clinical or overt nephropathy, macroalbuminuria is >300 microgms/ mg creatinine in the spot urine sample, or >500 mg/day in a 24 hour urine collection (3). Endstage renal

disease develops in 50% of type 1 diabetics with overt nephropathy within 10 years and in >75% by 20 years. Without specific interventions, 20 to 40 % of type 2 diabetics with microalbuminuria progress to overt nephropathy, but by 20 years after onset of overt nephropathy, only about 20% will have progressed to ESRD (3). Albuminuria is a marker of greatly increased cardiovascular morbidity and mortality for diabetic patients (3).

III. **Retinopathy**

It was found that diabetics are 25 times more likely to become legally blind than non diabetics (3). Diabetic retinopathy is characterized by microaneurysms, blot hemorrhages and cotton wool spots, and based on this is classified into non-proliferative and proliferative. Non-proliferative retinopathy is seen in about 25% of diabetics in 5 years and in 80% after 15 years among type 1 diabetics and in about 39% in type 2 diabetics (3).

IV. **Coronary artery disease**

Coronary artery disease is 3 times more common in diabetics. The usual manifestations include angina, acute myocardial infarction, post MI failure, dysrhythmias, complications like shock, conduction disturbances, cardiac failure, re-infarction and ketoacidosis. Other manifestations included are atypical ischemic symptoms like dyspnoea, hypotension, sweating and syncope etc (3).

V. **Cerebrovascular disease:**

Diabetics have also a higher incidence of cerebrovascular disease in the form of transient ischemic attack, stroke, carotid stenosis etc. The risk of stroke is at least three fold higher in diabetics than in nondiabetics (3).

VI. Peripheral vascular disease: -

It is clinically identified by a history of intermittent claudication and absent peripheral pulses in the extremities. With the use of doppler technology and blood pressure measurements of the extremity, it can be recognised noninvasively before clinical manifestations although angiography remains the gold standard. It is more than twice as common in the diabetic population as in the nondiabetic group, as evidenced from the ARIC and UKPDS studies (3).

Hypomagnesemia in Diabetes mellitus

Introduction

Magnesium has been proposed to play a role in the pathogenesis of diabetes and its complications.

To date, only obesity and physical inactivity have been well established as modifiable risk factors for type 2 diabetes. Magnesium deficiency has been emerging as a novel risk factor, which could be modifiable.

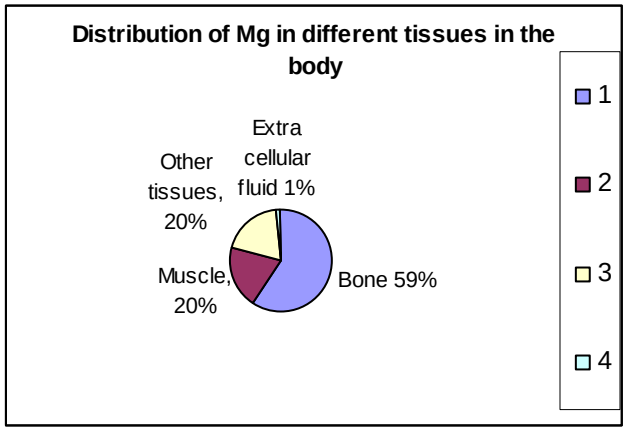
Magnesium (Mg^{2+}) is the second most abundant intracellular cation after potassium and the fourth most abundant cation of the body after calcium, potassium, and sodium. It has a molecular weight of 24.3 and the valence is +2, and 1 meq/L is equivalent to 0.50 mmol/L or 1.2 mg/dL. The normal range of plasma magnesium concentration is 1.7-2.4 mg/dl (0.7-1.0 mmol/l), and is maintained remarkably constant (3).

Biochemistry of Magnesium

Mg^{2+} is an important co-factor for many biologic processes, most of which use ATP. It is an essential mineral that is important for bone mineralization, muscular relaxation, neurotransmission and other cell functions. Most of the intracellular magnesium is located within the mitochondria apparently because magnesium binds strongly with ATP. In general, the more metabolically active the cell is, the higher is its magnesium content (16). Extracellular Mg^{2+} concentration is tightly regulated by the extent of intestinal absorption and renal excretion.

Body stores of magnesium

Dietary Mg content normally ranges from 140 to 360 mg/day (3) and the total body Mg2+ concentration is approximately 2000 mEq, or 25 g. The distribution of Mg2+ in the body is as follows:



1. Bone- about 60%
2. Muscle-20%
3. Other tissues- 20%
4. Only a small fraction-1% is in the extracellular fluid compartment. Because serum Mg is only <1% of total body Mg, measurements of serum Mg levels may not accurately reflect the levels of body stores of Mg (3, 17).

Magnesium homeostasis depends on the balance between intestinal absorption and renal excretion. Within physiological ranges, diminished magnesium intake is balanced by enhanced magnesium absorption in the intestine and reduced renal excretion. These transport processes are regulated by metabolic and hormonal influences, but these influences are not as significant as in calcium homeostasis (17)

Absorption and regulation of magnesium

A) Gastrointestinal Metabolism:

The principal site of magnesium absorption is the small intestine, with smaller amounts being absorbed in the colon. 25 to 60% of dietary Mg is absorbed in the gastrointestinal tract (2).

Intestinal magnesium absorption occurs via two different pathways (2):

1. A paracellular simple diffusion (passive transport) at high intraluminal concentrations.
2. A saturable active transcellular transport which involves Mg specific transporters, esp, TRPM6 (Transient receptor potential channel melastatin 6) along the brush border membrane of the small intestine at low intraluminal concentrations.

B) Renal Metabolism **(Figure 2):**

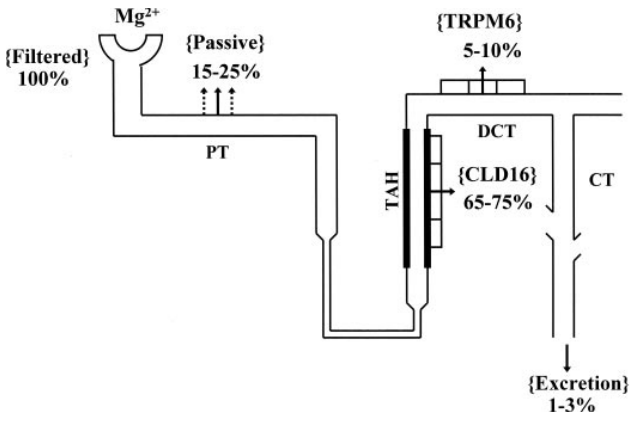
1. Glomerular filtration: In the kidney, 80% of total serum magnesium is filtered in the glomeruli with 95% being reabsorbed along the nephron (2).

2. Proximal tubules: 15–20% is reabsorbed in the proximal tubule.

3. Loop of Henle: 70% is reabsorbed in the loop of Henle, especially in the cortical thick ascending limb (TAL).

4. Distal convoluted tubules: Only 5–10% of the filtered magnesium is reabsorbed in the distal convoluted tubule (DCT). It determines the final urinary magnesium excretion.

5. Finally, 3–5% of the filtered magnesium is excreted in the urine..



(Figure2: Borrowed from Clin. J.Am.Soc. Nephrol. 2007 by Pham etal (2)
Renal Mg handling- After glomerular filtration, ionized Mg is reabsorbed passively in parallel to sodium reabsorption in 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); and CT- collecting tubules.)

Epidemiology of hypomagnesemia

1. Prevalence of hypomagnesemia in the general population:

Estimates of prevalence in the general population ranges from 2.5% to 15% in different studies (18). There are certain factors that are associated with a higher risk for hypomagnesemia:

a) elderly
b) people in nursing/ old age homes
c) hospitalized patients
d) decreased dietary intake- refined foods, acid rain
e) decreased drinking water content of magnesium

2. Prevalence of hypomagnesemia in the hospital population:

Hypomagnesemia is a common entity occurring in about 7 to 12% of hospitalized patients (16, 19). The factors for the high prevalence of hypomagnesemia in hospitalized patients could be

- Magnesium free parenteral fluids
- Prolonged nasogastric suction
- Infectious diarrhea
- Steatorrhoea
- Inflammatory bowel disease
- GI neoplasms.
- Drugs- diuretics
- Alcohol dependence- Incidence of hypomagnesemia among people with alcohol dependence is approximately 25% and mainly is due to magnesium diuresis caused by alcohol.
- Intensive care setting

3. Prevalence of hypomagnesemia in the Intensive care unit (ICU) population

Hypomagnesemia is extremely common in patients in ICU (20, 21) and the incidence rises to as high as 60 to 65% in patients in this setting. In one study hypomagnesemia on admission to the intensive-care unit was associated with a mortality rate approximately twice that of comparably ill normomagnesemic patients (22, 23). It has not been shown however, that treatment with magnesium supplementation would improve the outcome. Most of the ICU's are now supplementing Mg for their patients on a routine basis.

4. *Prevalence of Hypomagnesemia among Patients with Type 2 Diabetes*

Hypomagnesemia 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 (24-29). Magnesium has been associated with the cause and consequence of diabetes and its complications. There are many studies around the world, and a few from India, which have looked at the prevalence of diabetes in hypomagnesemic individuals. There are also studies which looked at correlation of diabetic complications with hypomagnesemia. Mg has also been implicated in the negative correlation between cardiovascular disease and hardness of drinking water. 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 (25, 30).

a) Prevalence studies from the west:-

There are many studies from the west which have shown a high prevalence of hypomagnesemia in diabetic populations, as given in table 3. Some of the studies have compared mean Mg levels between IDDM and NIDDM for eg. by Garber (31).

Chambers found a lower mean Mg concentration among the diabetics than in the nondiabetics, 1.92 ± 0.16 mg/dl in diabetics vs. 2.01 +/- 0.16 mg/dl in nondiabetics (p <0.001) as shown in table 3 (32).

Table 3: Hypomagnesemia- Studies on prevalence and mean Mg concentrations from abroad:

Study name, place and journal published (reference)	**Number of diabetics included**	**Mean magnesium value**	**Prevalence of hypomagnesemia**	**Relevant features**
Garber etal, United states Clin Ther 1996 (31)	199 diabetics, who had never been on Mg supplementation	1.48mg/dL in IDDM, and was 1.44mg/dL in NIDDM	78.3%	No correlation was found between HbA1c and serum Mg levels
Rodriguez-moran etal Mexico Diabetes Care 2003 (33)	63 diabetics	1.56 +/- 0.16 mg/dl	NA	Serum Mg inversely correlated with HbA1c values
Chambers etal New York J.Am.col Nut 2006 (32)	485 diabetics	1.92 ± 0.16 mg/dl vs. 2.01 +/- 0.16 mg/dl in nondiabetics (p <0.001)	NA	Strongly correlated diabetes and hypomagnesemia
Schnack etal Germany Diabetologia 1992 (34)	50 diabetics	1.89 +/- 0.02 mg/dl in DM vs 2.11 +/- 0.02 mg/dl in normal controls (p less than 0.0001).	NA	Serum Mg levels were low inspite of strict metabolic control over 3 months
Levin etal Germany Diabetologia 1981 (35)	17 diabetics and 17 normal	1.92 +/- 0.04 mg/dl in diabetics vs. 2.16 +/- 0.04 mg/dl in normals (p < 0.001)	**NA**	Intracellular Mg content was not different between the cases and controls
Khan etal Bangladesh Am J Clin Nut 1999 (36)	40 which included type 2 diabetes (I), Fibrocalculous diabetes mellitus (II) and protein deficient diabetes (III)	1.68+/-0.19 mg/dl in I, 1.58+/-0.16 mg/dl in II and 1.63+/-0.14 mg/dl in III	42.85% in I, 69.23% in II and 61.54% in III	Significantly lower Mg levels in the diabetics compared to normal controls

b) Studies from India showing prevalence of hypomagnesemia and mean Mg concentration in diabetics-

There are also a few studies from India- mainly from the north india, which have shown a higher prevalence of hypomagnesemia in the diabetics and also a lower mean Mg concentration in the diabetics than nondiabetics. Table 4 gives a summary of the studies published from the north India with regard to the same.

Table 4: Hypomagnesemia - Studies from India:

Study name, place and journal published (reference)	**Number of diabetics included**	**Mean magnesium value**	**Prevalence of hypomagnesemia**	**Relevant features**
Sharma Bikaner JIMA 2007 (37)	50 diabetics and 40 normals	1.93 +/- 0.282 meq/l in diabetics and 2.25 +/- 0.429 meq/l in controls ($p<0.005$)	**NA**	Strong association between hypomagnesaemia and retinopathy, obesity and hypertension
Hans Chandigarh Int J Diab Dev Ctries 2002 (38)	100 diabetics	1.81 +/- 0.12 mg/dl in diabetics Compared to 2.06+/-0.068 mg/dl in normals ($p<0.02$)	**25%**	Strong correlation between serum and RBC Mg, also with oxidative stress
J Lal New Delhi JAPI 2003 (39)	40 diabetics and 54 controls	1.44 +/- 0.48 mg/dl in cases Compared to 2.29 +/- 0.33mg/dl in controls ($p<0.001$)	**NA**	Supplemenattion showed improvement in lipid profile, but no significant effect on blood glucose levels

Hypomagnesemia and diabetic complications

There are also a few studies which have shown a positive correlation between hypomagnesemia and diabetic complications like cardiovascular disease (28), diabetic foot ulcers (7), retinopathy and neuropathy.

- *Coronary artery disease*- Hypomagnesemia has been for a long time associated with development of atherosclerosis and coronary artery disease. There are many follow up studies which have shown an association between the two including ARIC cohorts as published by Jing Ma etal and Liao etal. (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- found an inverse association between serum Mg and the risk for coronary heart disease in men with diabetes (28, 40)).

Magnesium depletion can induce changes in the electrocardiogram- widening of the QRS complex and peaking of T waves have been described with modest magnesium loss, while more severe magnesium depletion can lead to prolongation of the PR interval, progressive widening of the QRS complex, and diminution of the T wave (24, 41).

The clinical disturbance of greatest potential importance, however, is the association of mild hypomagnesemia with ventricular arrhythmias in patients with cardiac disease. A number of uncontrolled studies suggest that hypomagnesemia may be an important risk factor for arrhythmias in the setting of an acute ischemic event, congestive heart failure, torsades de pointes, after cardiopulmonary bypass, or in the acutely ill patient in the intensive care unit.

The mechanism underlying a possible association between hypomagnesemia and arrhythmias is at present unknown. Arrhythmias could be due to concurrent hypokalemia,

hypomagnesemia itself, or both. Magnesium regulates several cardiac ion channels, including the calcium channel and outward potassium currents through the delayed rectifier (42).

❖ *Diabetic Retinopathy*- The link between hypomagnesemia and diabetic retinopathy was also supported by multiple studies, and 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 (43). Mc Nair etal reported that diabetes induced damage to the eyes is more likely to occur in magnesium deficient patients with insulin dependent diabetes mellitus (44). A similar link, however, was not observed when Mg was measured within mononuclear cells.

❖ *Foot Ulcerations*- **G**iven the link between hypomagnesemia and risk factors for the development of diabetic foot ulcers (*e.g.*, polyneuropathy, platelet dysfunction), Rodriguez- Moran and Guerrero-Romero (7) suggested that hypomagnesemia may be associated with an increased risk of diabetic foot ulcers, as elucidated in table 5 below. Indeed, they observed a higher incidence of hypomagnesemia among their patients with diabetic foot ulcers compared with those without the condition and also a lower mean Mg concentration among the foot ulcer patients, than those without.

❖ *Nephropathy*- Hypomagnesemia has also been associated with microalbuminuria and nephropathy. It is a controversy, as to whether the hypomagnesemia could be in fact secondary to hypermagnesuria in nephropathy or whether hypomagnesemia is anyway linked to advanced diabetes with complicatins. Corsonello etal conducted a comparative study that involved 30 patients who had type 2 diabetes without microalbuminuria, 30

with microalbuminuria, and 30 with overt proteinuria, and observed a significant decrease in serum ionized Mg in both the microalbuminuria and overt proteinuria groups compared with the nonmicroalbuminuric group as shown in table 5 (45). According to a study done by Pham etal, there was a strong association between the low levels of serum magnesium and a faster rate of renal dysfunction among type 2 diabetics. Patients belonging to lower Mg groups for both genders had significantly worse slopes when plotted against renal parameters and that association was independent of the presence of hypertension and use of ACEI/ARB, diuretics, HMG-CoA enzyme inhibitors or aspirin (25).

- *Others- Dyslipidemia and hypertension*- Finally, there also are data to suggest the association between hypomagnesemia and other diabetic co-morbidities like dylipidemia (46) and hypertension (28), also showing improvement after supplementation (47).

Table 5: Hypomagnesemia- Correlation with dibetic complications and co-morbidities

Study name, place and journal published (reference)	**Number of diabetics included**	**Mean magnesium value**	**Prevalence of hypomagnesemia**	**Relevant features**
Rodriguez-moran etal Mexico Arch Med Res. 2001 (7)	33 diabetics with foot ulcers (cases) vs. 66 diabetics without foot ulcers (controls)	1.48 +/- 0.33 mg/dl in cases vs. 1.68 +/- 0.32 mg/dl in controls ($p<0.001$)	93.9% in cases vs. 73.1% in controls ($p=0.02$)	Hypomagnesmia had strong correlation with foot ulcers , odds ratio 2.9
Corsonello etal Italy Am J Nephrol (45)	30 diabetics with normoalbuminuria(I) 30with microalbuminuria (II) and 30 with clinical proteinuria (III)	Serum ionized Mg levels- 1.08+/-0.04 mg/dl in group I; 0.86 +/- 0.12 mg/dl in group II; 0.84 +/- 0.09 mg/dl, $p < 0.001$	**NA**	Serum ionized Mg levels seems to positively correlate with stages of diabetic nephropathy
Lima etal, Brazil Diabetes care 1998 (29)	128 diabetics	1.77+/-0.41 mg/dll	47.7% total serum Mg, 31.1% had low intramononuclear levels	Positively correlated with peripheral neuropathy and CAD

Evidence for benefit from supplementation

A few studies had shown benefit from replacement of magnesium in various aspects (Table 6), including improvement in co-morbidities- dyslipidemia and hypertension. Most of the intervention trials have been done on small populations of diabetics. The duration for supplementation has also been of the range of few weeks to months, and there is lack of evidence for benefit from long term supplementation. In the trials, both high concentration Mg solutions and Mg tablets have been used for supplementation.

Table 6: Studies - Benefit from Mg supplementation

Study name and journal published	Number of diabetics included	Compound used	Duration of treatment	Change in serum magnesium	Outcomes
Yokota etal, Japan Journal of the American Coll of Nutr 2004 (48)	9	MAG21, viscous acidic water with MG content 71mg/dl	30 days	2.1 +/- 0.06 mg/dl to 2.3 +/- 0.07 mg/dl	a)Systolic BP- 136.3+/-2.68 to 130.8+/-2.79 b) DBP -83.0+/-5.14 to 79+/-5.86 c)Triglycerides from 255.4+/-80.5 to 178.8+/-38.3
Rodriguez Moran etal Mexico Diabetes care, 2003 (33)	63	50 ml MgCl2 solution and placebo	16 weeks	1.56+/-0.17 mg/dl vs 1.77+/-0.24 mg/dl in controls	a) HbA1c 8.0+/-2.4 vs. 10.1+/-3.3% in the controls b) Total cholesterol 6.4+/-1.9 vs. 7.0+/-1.7
deValk etal, Netherlands Diabet Med 1998 (49)	50	15 mmol of Mg vs placebo	3 months	Plasma Mg after supplementation (1.96 +/- 0.17mg/dl in cases vs 1.87 +/- 0.19 mg/dl, p < 0.05), in placebo	Increase in urinary Mg excretion, no change in glycemic control or lipid concentration
Eibl etal United States Diabetes care 1995(50)	40	30 mmol of oral Mg/day	3 months	Mg: 1.75 +/- 0.19 mg/dl baseline vs. 1.94 +/- 0.02 mg/dl after supplementation 2.11 +/- 0.19 mg/dl in controls (p-NS)	Metabolic control was not altered
J Lal etal, Delhi JAPI 2003 (39)	40	600 mg of MgO once a day	12 weeks	1.44 +/- 0.48 mg/dl to 2.17 +/- 0.26 mg/dl	a)Triglycerides from 189.3+/-93.8 to 153.8+/-53.5 b)LDL from 124.1+/-53.3 to 76.1+/-27.3 c)Diastolic BP from 76.55+/-8.66 to 71.1+/-6.93 mm of Hg

Hypomagnesemia – Possible mechanisms in the pathogenesis of diabetes and diabetic complications

There is considerable evidence to suggest that hypomagnesemia may adversely affect various aspects of cellular physiology. Insulin regulates the intracellular magnesium concentration by stimulating the plasma membrane ATPase pump (51).

1) Insulin resistance- A study to explore link between magnesium deficiency and insulin resistance, looked at the effect of diet lacking in magnesium on insulin resistance in non diabetics (52). Diet induced magnesium deficiency leads to decreased insulin sensitivity in lean non-diabetics (53). It was also shown that oral magnesium supplementation improves insulin sensitivity in hypomagnesemic non-diabetic subjects (6).

2) Accelerated atherosclerosis- *Hypomagnesemia has been proposed to accelerate atherosclerosis via the following mechanisms:*

a) *Endothelial dysfunction and thrombogenesis*- It may promote endothelial cell dysfunction and thrombogenesis *via* increased platelet aggregation and vascular calcifications (35, 54, 55). It causes collagen and ADP induced platelet aggregability, by increasing the pro-aggregating effect of leptin (38, 56).

b) *Reduction in plasma antioxidant levels*

- Plasma levels of malondialdehyde have been found to be increased in the diabetic patients with hypomagnesemia, associated with significant reduction in the levels of plasma antioxidants (38).

-Magnesium deficiency may be involved in the initiation and propagation of free radical myocardial tissue damage through oxidation of myoglobin, which is essential for intracellular transport and storage of oxygen (17, 39, 57).

- Hypomagnesemia causes decreased ascorbic acid levels.

- Magnesium is an obligatory cofactor in the enzyme reaction of GSH synthesis and so magnesium deficiency has been reported to inhibit the biosynthesis of GSH (58, 59)

c) *Proinflammatory and Profibrogenic response*- It may lead to the induction of proinflammatory and profibrogenic response (36-38, 60).

d) *Induction of vasoconstriction and hypertension*- By elevation in smooth muscle and cardiac intracellular calcium concentration (61). It has been observed that after Mg supplementation, there is increase in intracellular Mg and decrease in intracellular sodium, thus lowering blood pressure through the suppression of adrenergic tone and possible natriuresis, because Mg supplementation increases urinary noradrenaline excretion (62).

e) *Reduction of inositol transport*- A reduction in the rate of inositol transport, and subsequent intracellular depletion was also linked to the development of complications (63).

f) *Increase in urinary thromboxane concentration* – It was observed that the urinary thromboxane concentration increased after magnesium deficiency, pointing towards hypomagnesemia as a common factor in insulin resistance and vascular disease (53).

Causes of hypomagnesemia

It has been observed that refining and processing of food causes a substantial loss of magnesium. For example, the refining and processing of wheat to flour, rice to polished rice and corn to starch depletes magnesium by 82, 83 and 97% respectively. Thus a western lifestyle and processing partially explains why a significant segment of the population has intake of magnesium below recommended dietary amounts and may be predisposed to chronic, latent magnesium deficiency (64).

The western diet is characterized by a high intake of saturated and trans fats and refined grains and low intake of whole grains, vegetables and fiber, resulting in low micronutrient intake(65).

There are other factors which have reduced magnesium within the ecosystem as a whole. Acid rain causes exchange between magnesium and aluminium in the soil. This coupled with intensive farming of the soil, has led to a reduction in magnesium within the food chain **(**1)**.**

The contribution of drinking water magnesium to overall magnesium intake is generally about 10% (66). It has been argued that bioavailability of magnesium is greater from water than from food, and there are studies which have shown that upto 40-50% of waterborne magnesium may be absorbed compared to about 30% from food (67). Thus waterborne magnesium would have relative importance, particularly in patients whose diets are relatively deficient in magnesium. The usual causes for hypomagnesemia are given in table 7.

Table 7: Causes of hypomagnesemia (3, 68):-

a) Poor Magnesium intake - Starvation - Anorexia - Protein calorie malnutrition - No Mg in intravenous fluids	b) Gastrointestinal losses - Nasogastric suction - Vomiting - Intestinal bypass for obesity - Short bowel syndrome - Inflammatory bowel disease - Pancreatitis - Diarrhea - Laxative abuse - Villous adenoma -Primary infantile hypomagnesemia
c) Renal losses - Acute renal failure- diuretic phase of acute tubular necrosis - Renal tubular acidosis - Post obstructive diuresis - Primary renal tubular magnesium wasting - Bartter syndrome - Gitelman syndrome	d) Drugs - Diuretics- Acetazolamide, thiazides, furosemide - Alcohol - Antibiotics-Aminoglycosides, Carbenicillin - Cisplatin - Digoxin - Ethacrynic acid - Foscarnet - Mannitol - Methotrexate - Pentamidine - Tacrolimus
e) Endocrine disorders - Hyperaldosteronism - Hyperparathyroidism - Hyperthyroidism - Diabetes mellitus - Hypoparathyroidism - Syndrome of Inappropriate secretion of Anti diuretic hormone	f) Other causes - Alcohol withdrawal - Extensive Burns - Exchange transfusions (redistribution) - Lactation - Acute pancreatitis (redistribution)

Possible causes of hypomagnesemia in diabetes

The following are the possible explanations for the higher prevalence of hypomagnesemia in diabetics (2), as modified from Pham etal, in Clin J Am Soc Nephrol: -

1. *Dietary deficiency* – The major sources of magnesium in the food supply are dairy products (20%), grain products (20%), meat, poultry and fish (15%), legumes, nuts and soya products (13%)- most of these have been restricted in a diet prescribed for diabetics, which could be contributing to the higher prevalence of hypomagnesemia in diabetics (28).

2. *Decreased intake* – secondary to diabetic autonomic neuropathies, which may reduceintake and absorption- (69):

-poor oral intake

-esophageal dysfunction

-diabetic gastroparesis

3. *Enhanced gastrointestinal loss*

-diarrhea as a result of autonomic dysfunction

4. *Enhanced renal magnesium loss*

-enhanced filtered load by glomerular hyperfiltration

-osmotic diuresis (glucosuria)

-metabolic acidosis (diabetic ketoacidosis)- by increasing the serum ionised Mg fraction (70)

-hypoalbuminemia- by increasing the serum ionised Mg fraction (70)

-microalbuminuria and overt proteinuria- as a result of protein bound magnesium loss (45)

5. *Enhanced tubular flow*

-volume expansion as a result of excessive volume replacement- induce renal Mg wasting and may reduce tubular reabsorption due to high tubular flow (70).

6. *Reduced renal reabsorption*

-endocrinologic dysfunction: insulin deficiency or resistance, causing decreased Mg reabsorption at the TAL (71)

-metabolic acidosis (diabetic ketoacidosis)

-diuretics (2)

Clinical manifestations of hypomagnesemia

Hypomagnesemia may cause generalized alterations in neuromuscular functions as described in the following table 8 (3). They are usually aymptomatic when serum Mg levels are greater than 1.2 mg/dl.

Table 8: Manifestations of hypomagnesemia

a)Electrolyte abnormalities	***b)Neuromuscular***	***c) Cardiovascular***
hypokalemia hypocalcemia	carpopedal spasm tetany muscle cramps fasciculations weakness tremulousness hyperactive reflexes myoclonus	ventricular arrhythmias torsade de points supraventricular tachycardia enhanced sensitivity to digoxin angina pectoris congestive heart failure atherogenesis

d) Neurologic vertigo ataxia nystagmus aphasia hemiparesis depression psychosis delirium choreoathetosis		***e) Skeletal*** Osteoporosis Osteomalacia

Diagnosis of Hypomagnesemia

Hypomagnesemia is diagnosed when the serum level of magnesium value is less than or equal to 1.7 mg/dl or less than 0.7 mmol/l or <2 SD below the mean of the general population (3).

Traditionally, hypomagnesemia refers to a low serum magnesium (Mg) concentration because this measurement has long been readily available. However, because Mg is mostly an intracellular cation, it has been questioned whether one can use measurements of serum Mg concentrations to study the impact of Mg on various physiologic conditions. Some investigators, instead, have used measurements of intracellular Mg concentrations, which is usually estimated using NMR (Nuclear magnetic Resonance) spectroscopy. The usual cells where the intracellular levels are measured are- erythrocytes or lymphocytes (72). Some studies have been done on muscle and brain- and it has been measured non-invasively by NMR spectroscopy. Clinically, it has been suggested that in a patient with suspected Mg deficiency, a low

serum Mg concentration is sufficient to confirm the diagnosis. However, if the serum Mg level is normal in this patient, then other more sensitive tests – intracellular (54, 73) and ionized levels should be performed. Although controversies still exist as to how hypomagnesemia could be best gauged, most of the studies so far have relied predominantly on the measurement of serum Mg concentrations. There are a few studies done using serum ionised magnesium concentration (the cut off is 0.46 mmol/l or 1.1 mg/dl) which also showed significantly lower levels of serum ionized Mg in diabetics (45, 74, 75).

The diagnosis can usually be obtained from the history, as magnesium depletion is a result of either gastrointestinal or renal losses, often promoted by drugs. In less obvious cases, the distinction between gastrointestinal and renal losses can be made by determination of the fractional excretion of magnesium or the measurement of the magnesium excretion in the 24-hour urine sample (68). The fractional excretion is calculated as follows

$$FE.\ Mg = \frac{U.Mg \times P.Cr}{(0.7 \times P.Mg) \times U.Cr} \times 100$$

(*U and P refer to the urine and plasma concentrations of magnesium (Mg) and creatinine (Cr). About 70% of PMg are not bound to albumin and therefore, filtered across the glomerulus (0.7 X PMg))*

Fractional excretion above 2% in a subject with normal renal function indicates renal magnesium wasting (68). The reference range of 24 hour urine magnesium is 3-5 mmol/l, ie equivalent to 70- 120 mg/l (76) .

Treatment of hypomagnesemia

The route of magnesium repletion depends on the severity of clinical manifestations. In cases of severe (<1.2mg/dl in the serum) and symptomatic hypomagnesemia with neuromuscular or neurologic manifestations or cardiac arrhythmias, Mg repletion should be achieved by intravenous administration of 2 g of Magnesium sulphate in 100 ml of 5% dextrose over 5 to 10 min and followed by a continuous infusion of 4 to 6 g/d for 3 to 5 d if renal function is relatively normal. The indication for parenteral administration is symptomatic hypomagnesaemia with tetany or severe ventricular arrhythmia (68). The underlying disease should be treated, if possible.

Correcting the hypomagnesemia, especially by intravenous application, will partially remove the stimulus to magnesium retention in the kidney as the plasma magnesium concentration is the major regulator of active magnesium resorption in the loop of Henle. Thus, oral supplementation is preferred in symptom-free patients (5– 20 mmol/day in divided doses as magnesium chloride or lactate). However, diarrhea may become a dose-limiting side-effect. Maintenance therapy may require oral administration of Mg oxide (400 mg twice daily or three times daily) for as long as the risk factors for Mg deficiency exist. Oral Mg gluconate (500 mg twice daily or three times daily) can also be used (77).

Hypomagnesemia and other electrolyte abnormalities

Associated hypokalemia

Hypokalemia is a common event in hypomagnesemic patients, occurring in 40 to 60% of cases (78). This relationship is in part due to underlying disorders that cause both magnesium and potassium loss, such as diuretic therapy and diarrhea. Potassium secretion from the cell into the lumen in the cells of the thick ascending limb and cortical collecting tubule is mediated by ATP-inhibitable luminal potassium channels (79). Hypomagnesemia is associated with a reduction in cell magnesium concentration, which may then lead to a decline in ATP activity, and, due to removal of ATP inhibition, an increase in the number of open potassium channels (79).

Associated Hypocalcemia

The most classical sign of severe hypomagnesemia <1.2 mg/dl is hypocalcemia.

A reduction in extracellular magnesium concentration stimulates the secretion of parathyroid hormone (PTH) in the absence of changes in calcium concentration (80, 81).

The adenylate cyclase systems of various organs may be affected differentially by a state of magnesium deficiency. It is suggested that magnesium deficiency may result in defective cyclic AMP generation in the parathyroid glands and in the PTH target organs. This could be the principal mechanism operative in both impaired PTH secretion and end-organ resistance to PTH which together contribute to the development of hypocalcaemia (81).

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METHODOLOGY

Setting The study was conducted among the patients presenting to the diabetic outpatient clinic run by the Department of Endocrinology of the Christian Medical College (CMC), Vellore, South India, which is a 2200 bedded tertiary care teaching hospital.

Duration of Study

May 2007 to May 2008.

Inclusion Criteria

1. Diabetic patients who were ambulant and willing to complete a 24 hour urine and serum sample were included after informed consent.
2. Diabetics who were willing to follow up in future in the same outpatient clinic- belonging to areas within a 50 km radius from the main hospital

Exclusion Criteria

1. The patients who had not completed the investigations – serum magnesium and 24 hours urine magnesium.
2. Diabetics who were not willing to follow up in the same out patient clinic

Study Design

The study was a cross-sectional descriptive study on diabetic patients attending the endocrine diabetic clinic in our centre.

Sample size

With an expected incidence of 25% and absolute precision of 10 % with an alpha error of 5%, the desired sample size calculated was 80.

Study Protocol

All local patients who were diagnosed to have diabetes mellitus, and were attending the diabetic endocrine clinic were interviewed. The patients who fulfilled the inclusion criteria, and were willing to participate in the study, were enrolled into the study. Upon enrollement into the study, complete demographic details, relevant clinical and laboratory parameters were collected. The details including duration of diabetes, drug history and parameters for comorbidities were also collected.

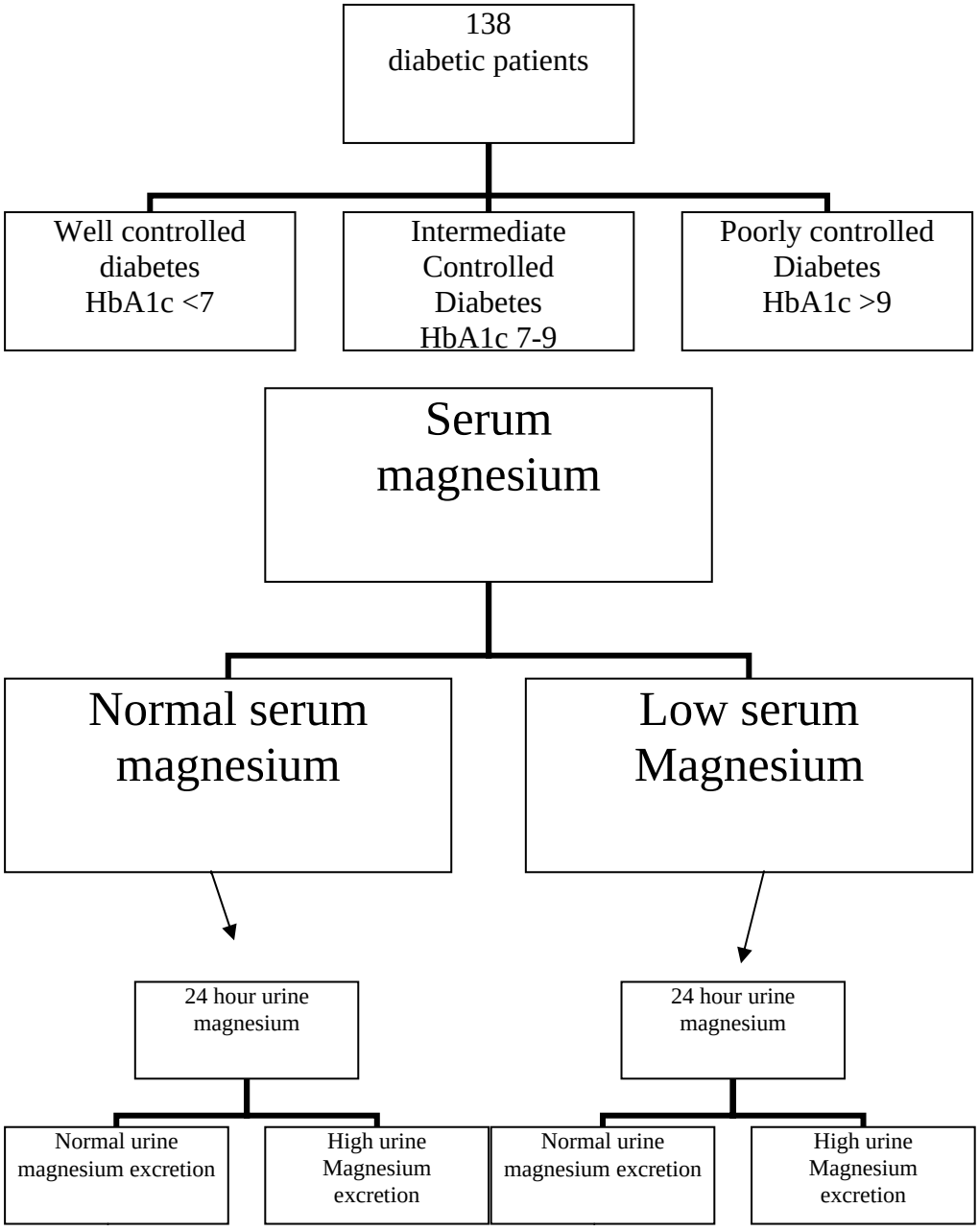
The following specific data was collected through a proforma (Annexure A) at the time of enrollment into the study

1. Demography- Age, Sex, Region, Marital status, Phone number
2. Evaluation of stage of diabetes- duration
3. Vital parameters- blood pressure, body mass index
4. Clinical evaluation of diabetes- including evaluation for hypertension, retinopathy, neuropathy by monofilament and

biothesiometer, peripheral vascular disease by absent pulses, carotid and renal artery stenosis- by bruit.

5. Laboratory parameters- regarding glycemic control by HbA1c, lipid profile- Total, LDL and HDL cholesterol, evaluation for nephropathy by urine microalbumin/ 24 hour urine protein/ serum creatinine.
6. Drug history – Details of treatment for diabetes- including sulphonylureas, biguanides, insulin, thiazolidinediones and others either single or in combination was documented. Also the use of drugs which could influence Mg homeostasis- diuretics was also documented.
7. The Magnesium evaluation included- a serum Magnesium sample and 24 hour urine magnesium by atomic absorption spectrophotometry.
8. A dietary history by a 24 hour recall was done to estimate the approximate daily consumption of magnesium for 14 patients. The dietary Mg intake was computed by multiplying the Mg content of the specified serving of each food item by the frequency of its daily consumption and summing overall items. (Annexure B).
9. Also the drinking water content of magnesium was estimated for 10 patients, by atomic absorption spectrophotometry.

CONSORT DIAGRAM



Definitions:

The following were the cut offs taken for defining the groups:

1. Definition of diabetes mellitus:-

- **Fasting ≥ 126mg% (Fasting is defined as no calorie intake for atleast 8 hours)**
- **2 hours Postprandial glucose ≥ 200mg%**
- **symptoms of diabetes plus Random Blood Glucose≥200mg%**

2. Glycemic control: - HbA1c <7- was taken as good control

- HbA1c >7 were divided into the following groups

-7-8.9- group1

-9-10.9- group 2

-11-12.9- group 3

->13- group 4

3. Hypertension: - defined as a) systolic blood pressure more than 140 mm of Hg

b) diastolic blood pressure more than 90 mm of Hg

c) patient who had been on antihypertensive drugs

4. Dyslipidemia- If LDL was >100mg/dl

Or Triglycerides >150mg/dl

5. Body mass Index- defined as a) Malnourished if BMI was less than 18 kg/m2

b) Normal if BMI was between 18 and 25 kg/m2

c) Overweight if BMI was between 25 and 30 kg/m2

d) Obese if BMI was more than 30 kg/m2

6. Peripheral neuropathy- If Monofilament threshold >/= 4gm, in either of the feet

- If biothesiometer threshold was >/= 25 mV

Semmes- Weinstein Monofilament

Light touch sensation was tested using 2gm, 4gm and 10 gm monofilaments over metatarsal head/ ball of the great toe (while testing; only mild pressure was applied so that the filament was not bent). Loss of sensation over 2gm was considered as peripheral neuropathy in the lower limbs. Loss of protective sensation in the lower limb is indicated by 10 gms loss of sensation. The patient is asked to say “yes” each time he or she feels the filament. Failure to feel the filament at four of ten sites is 97% sensitive and 83% specific for identifying loss of protective sensation. This method has sufficient reproducibility, when used as screening test for diabetic foot ulcerations.

Biothesiometer- Quantitative Sensory Testing

It provides a quick and reliable assessment of vibration thresholds, which gives an objective measure of the progress of diabetic peripheral neuropathy. However, detection of impairment of vibration sense using tuning fork (128 Hz) is adequate, when a biothesiometer is not available. If the value is above 25V, that could be taken as evidence of neuropathy.

7. Nephropathy- Microalbuminuria was present if the urine microalbumin was between 30 and 300 microgm/mg of creatinine or 30- 300 mg/day in a 24 hour urine collection.

- Overt nephropathy/macroproteinuria– If urine microalbumin >300 microgm/mg of creatinine or 24 hour urine protein was more than 500mg/24 hours, or if there was evidence of renal failure as evidenced by raised serum creatinine (3)

8. Retinopathy- Present or absent as confirmed by an Ophthalmologist and the diagnosis was made in the presence of microaneurysms, dot and blot hemorrhages and evidence of macular edema, or any patient who LASER/ intervention for retinal detachment/ vitreous hemorrhage.

9**.** Cardiovascular disease- Any of the following features qualified:

a) Past history of acute coronary syndrome

b) Stable angina

c) History of PTCA/ CABG

d) TMT positivity

10. Cerebrovascular disease- Any of the following features

a) History of transient ischemic attack/ stroke

b) Carotid stenosis- either carotid bruit or Doppler proven

11. Peripheral vascular disease: - Any of the following features

a) Absent peripheral pulses

b) Claudication pain

c) History of gangrene/ amputation

12. Renovascular disease: - Any of the following features

a) Renal bruit

b) Doppler evidence of renal artery stenosis

13. Hypomagnesemia- was defined as a serum magnesium level less than or equal to 1.7 mg/dl.

14. 24 hour urine magnesium – The normal range was taken as 70 to 120 mg/dl, and any value more than 120 mg/dl was taken as inappropriate magnesuria.

15. Dietary content of Magnesium- The recommended daily allowance was 320 mg/day for women and 420 mg/day for men as per Krause's text book of food, nutrition and diet therapy (82).

16. Magnesium content of drinking water- At least 10mg/dl of magnesium content was considered adequate.

Magnesium estimation

Magnesium estimation was done by the Atomic Absorption Spectrophotometry released by the Perkin- Elmer Corporation in September 1996.

Analysis- For the determination of magnesium, dilute the serum or plasma sample in a 1:100 ratio, with deionised water as the diluent.

Principle- The machine consists of a sample introduction system, an excitation source (hollow magnesium lamp), nebuliser and flame burner, chopper and detector. An atom of magnesium is capable of absorbing light energy characteristic of magnesium. The radiation generated from the hollow magnesium lamp, whose cathode is made of magnesium is made to pass through the flame containing magnesium and the photons are absorbed. The degree of absorption is proportional to the concentration of magnesium in the flame (the flame also serves as the means of supporting the atoms in the light path). The measured difference between the light intensity passing around the flame and that passing through the flame defines absorption and is used to determine the concentration of magnesium in the atomized solution (83).

The magnesium standard used which was used in our study was 5 mg/l, and a QC (quality control) was estimated on a daily basis. The mean QC was 2.3 and the mean coefficient of variation was 1.8 during the time period of this study.

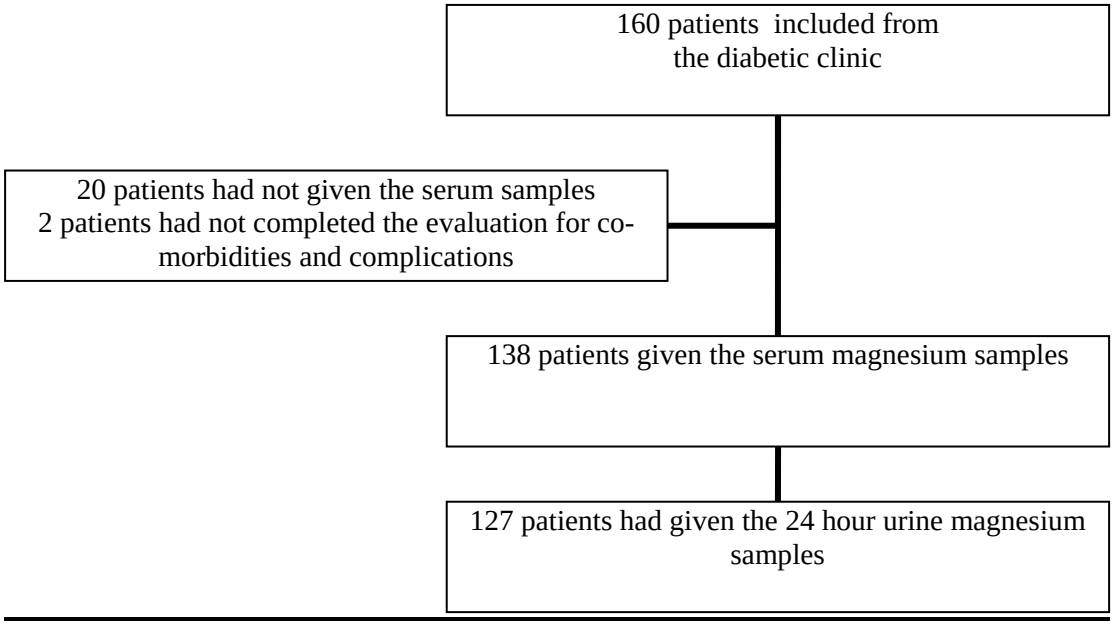
Statistical analysis

Continuous variables are presented using mean ± standard deviation and categorical variables are presented using frequencies and percentages. Continuous variables were compared using Student's t test. Association between categorical variables was assessed using Chi-square test with Yates continuity correction. All statistical analyses were performed using SPSS 11.0 for windows. A p-value < 0.05 was considered to be statistically significant.

RESULTS

A total of 138 diabetic patients were evaluated for the prevalence of hypomagnesemia between March 2008 and May 2008. All were diabetics who were attending the diabetic endocrine out patient clinic at the Christian Medical College Hospital (CMCH), Vellore. All of them had a complete evaluation for diabetic co-morbidities and complications- both microvascular and macrovascular. Of these, 126 patients had given 24 hour urine urine magnesium samples, to look for inappropriate magnesuria. A careful dietary history was taken for about 20 of the patients and drinking water magnesium content was estimated in 4 of the hypomagnesemic and 6 normomagnesemic patients. All the patients who had low serum magnesium, were informed about their low serum magnesium levels via phone, and the need for supplementation. The baseline characteristics of the patients, are included in table 1.

Fig 1. Patient recruitment for study



Baseline characteristics:

The baseline characteristics of the patients are presented in Table 9. Of the population, 52.9 % of the patients were male. Most of the patients- 51% were between 40 and 60 years age. The mean HbA1c for the whole group was 8.3+/- 1.74 g%. About 19% were well controlled diabetics, with HbA1c less than 7g%, and about 33% were poorly controlled diabetics with HbA1c more than 9%, as shown in graph 2. The mean BMI of the group was 25.4. Almost 53% were overweight, and about 46% had normal BMI. There were patients between 22 and 78 years age, and the mean age of the group was about 52.5. The mean duration of diabetes was 85.2 months (with a standard deviation of 76.6). About 50% of the population had been having diabetes for less than 5 years. Among the patients, 42 % were hypertensive and 77% had dyslipidemia.

With regard to the profile of diabetic complications, 35% had neuropathy, 45% had evidence of microalbuminuria and 17% had retinopathy (given in table 10). 10 % of the patients had frank nephropathy as evidenced by either renal failure or macroalbuminuria. Among the macrovascular complications, ischemic heart disease was seen in 13 % of the patients, only 1.4 % had evidence of cerebrovascular disease and none had peripheral vascular disease.

Most of the patients had been on oral hypoglycemic drugs- mainly sulphonylureas and biguanides, and some were on insulin in combination with the oral drugs. 66% were on sulphonylureas, 75% were on metformin (biguanides), and 29% were on insulin (as given in table 11).

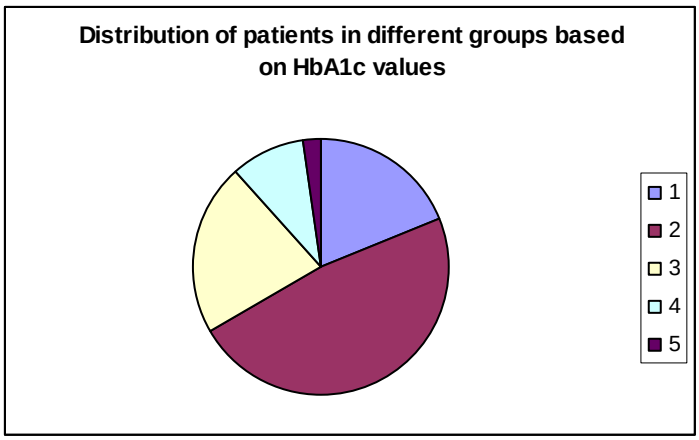
Among the drugs which are known to affect magnesium metabolism- diuretics and statins were widely used, (about 12% and 36% respectively).

Most of the patients were following the dietary instructions, as advised by the dietician in the diabetic out patient clinic. Most of the population depended on the government water supply for drinking water, and a minority depended on mineral water (processed water).

Table 9. Baseline characteristics of all the study patients

Baseline characteristics	**All patients**
Total number	138
Sex	
Male	73 (52.9%)
Female	65 (47.1%)
Age (mean in years)	52.5+/-11.9 (range from 22 to 78)
Age categories:	23 (16.7%)
20-40 yrs	71 (51.4%)
40-60 yrs	44 (31.9%)
60-80 yrs	25.4 +/-3.9 kg/m2
BMI (mean)	
BMI in subgroups (%)	2 (1.4%)
<18	63 (45.7%)
18-25	73 (52.9%)
>25	8.30 +/- 1.74
HbA1c (mean)	
HbA1C groups	26 (18.8%)
<7	66 (47.8%)
7-9	30 (21.7%)
9-11	13 (9.4%)
11-13	3 (2.2%)
>13	85.2 +/-76.6
Duration of diabetes mellitus in months	
Duration categories:	74 (53.6%)
Upto 60 months	31 (22.5%)
61-120 months	26 (18.8%)
121- 240 months	7 (5.1%)
> 241 months	

Graph 2; The distribution of diabetic patients in different HbA1C groups is as follows:



1.HbA1c <7g%

2.HbA1c 7-9g%

3.HbA1c 9-11g%

4.HbA1c 11-13g%

5.HbA1c >13g%

Table 10: Diabetic complications and co-morbidities

Co-morbidities and complications	**Total number of patients**	**Confidence intervals**
Co-morbidities		
Hypertension	58 (42%)	33.77, 50.23
Dyslipidemia	107 (77.5%)	70.53, 84.47
Diabetic microvascular complications		
-Microalbuminuria	62 (44.9%)	36.6, 53.2
-Nephropathy	14 (10.1%)	5.07, 15.13
-Neuropathy by monofilament	49 (35.5%)	27.51, 43.48
-Neuropathy by biothesiometer	23 (16.7%)	10.48, 22.92
-Motor neuropathy	33 (23.9%)	16.78, 31.02
-Retinopathy	24 (17.4%)	11.07, 23.73
Diabetic macrovascular complications		
-Ischemic heart disease	18 (13%)	7.39, 18.61
-Cerebrovascular disease	2 (1.4%)	0, 3.36
-Peripheral vascular disease	0	
-Renovascular disease	1 (0.7%)	0, 2.09

Table 11. Drug profile of the study population

Drug	No of patients (%)	Confidence intervals
Diabetic drugs		
Sulphonylureas	92 (66.7%)	58.84, 74.56
Biguanides	104 (75.4%)	68.21, 82.59
Thiazolidinediones	9 (6.5%)	
Meglinides	0	
Alpha glucosidase inhibitor	1 (0.7%)	0, 2.09
Insulin	40 (29%)	21.43, 36.57
Other drugs which could affect magnesium excretion		
Antibiotics- Aminoglycosides	0	
a. Carbenicillin	0	
b. Amphotericin	0	
Diuretics	17 (12.3%)	6.82, 17.78
Alcohol	2 (1.4%)	0, 3.36
Cisplatin	0	
Digoxin	2 (1.4%)	0, 3.36
Methotrexate	0	
Statin	50 (36.2%)	28.18, 44.22
Theophylline	1 (0.7%)	0, 2.09

Prevalence of hypomagnesemia:

A total of 7 patients out of 138, had hypomagnesemia with a prevalence of 5.1% (CI- 1.43 , 8.77%). The profile of the hypomagnesemic patients is described in table 12.

There were 7 diabetics with hypomagnesemia, with serum levels less than 1.7 mg/dl. The lowest serum Mg level observed in this group was 1.2 mg/dl. They did not have any features of cardiovascular and neuromuscular manifestations such as palpitations, documented arrhythmias, tremors and other abnormal movements. All of them had HbA1c levels more than 7g%, of which 4 were poorly controlled group as defined by HbA1c value more than 9g%. Their mean HbA1c value was 8.98 +/- 1.37 g%. Their mean duration of diabetes was about 83 months. All 7 of them had dyslipidemia, as evidenced by LDL > 100g% and triglycerides >150g%, and about 42% had hypertension.

Of the 7 patients, microalbuminuria and retinopathy was seen in about 42% of the group, and nephropathy, neuropathy as evidenced by monofilament and motor neuropathy was seen in about 28%. Among the macrovascular complications, ischemic heart disease was seen in 2 patients(28%). The only patient in the whole population who had renal vascular disease had hypomagnesemia.

Out of the 7 hypomagnesemic patients, 6 had been on insulin, and 5 had been on sulphonylureas and metformin. Among the drugs which could affect magnesium metabolism, 3 had been on statins and 1 had been on a diuretic.

Table 12: Profile of patients with hypomagnesemia

Characteristics	***Number (%)***
Sex	
Male	4 (57.1%)
Female	3 (42.9%)
Age (mean in years)	56.57 +/-16.65
<40 yrs	1 (14.3%)
>40 yrs	6 (85.7%)
BMI (mean)	24.17 +/-3.22
BMI in subgroups (%)	
<18	0
18-25	4 (57.1%)
>25	3 (42.9%)
HbA1c (mean)	8.98 +/-1.37
HbA1C groups	
<7	0
7-9	3 (42.9%)
9-11	3 (42.9%)
11-13	1 (14.3%)
>13	0
Duration of diabetes mellitus in months	113.29 +/-56.74
<60 months	1 (14.3%)
60-120 months	3 (42.9%)
121-240 months	3 (42.9%)
>240 months	0
Hypertension	3 (42.9%)
Dyslipidemia	7 (100%)
Diabetic complications	
-Microalbuminuria	3 (42.9%)
-Nephropathy	2 (28.6%)
-Neuropathy by monofilament	2 (28.6%)
-Neuropathy by biothesiometer	1 (14.3%)
-Motor neuropathy	2 (28.6%)
-Retinopathy	3 (42.9%)
-Ischemic heart disease	2 (28.6%)
-Cerebrovascular disease	0
-Peripheral vascular disease	0
-Renovascular disease	1 (14.3%)

Predictive factors for hypomagnesemia in diabetes

The baseline characteristics and the drug history among patients with and without hypomagnesemia are outlined in tables 13 and 14.

Baseline characteristics were compared among patients with and without hypomagnesemia. There was no significant difference in age, gender and body mass index (BMI) values between the 2 groups. Though the HbA1c was 8.98 in the hypomagnesemic group, which was higher compared to 8.3 in the normomagnesemic group, the difference was not statistically significant (p value 0.31). All the hypomagnesemics had HbA1c values more than 7, of which 57% had HbA1c more than 9, as compared to the proportion of patients who were normomagnesemic, although this difference was not significant. Though the duration of diabetes was shorter in the hypomagnesemics, compared to the normals, (83.6 months vs. 113.2 months), it was not statistically significant (p value= 0.32).

The proportion of patients with hypertension was comparable in both the groups. Although 100% of the hypomagnesemics were dyslipidemic, and about 76% among the normomagnesemics were dyslipidemic, the difference was not statistically significant (p value=0.14). The details with regard to co-morbidities and complications are given in table 14.

With regard to the diabetic complications, microalbuminuria was present in about 42.9% among the hypomagnesemics, and 45% among the normomagnesemics, and this difference was not statistically different (p=0.91). Among the hypomagnesemics, 28.6% had nephropathy and among the normomagnesemics, 9.2% had nephropathy, however this difference was approaching significance (p=0.09). Retinopathy prevalence among the

hypomagnesemics and normomagnesemics was 42% and 16% respectively, and this difference was also approaching statistical significance (p=0.07). There was also no significant difference among the 2 groups, with regard to neuropathy. Since the overall number of patients with cerebrovascular disease and renovascular disease was only 2 and 1 respectively, the tests of statistical significance were not applied. The only patient with renovascular disease had hypomagnesemia.

Table 13: Baseline characteristics of patients with and without hypomagnesemia

Baseline characteristics	**Patients with hypomagnesemia**	**Patients without hypomagnesemia**	**P value**
Total number	**7**	**131**	
Sex			
Male (n=73)	4 (57.1%)	69 (52.7%)	0.82
Female (n=65)	3 (42.9%)	62 (47.3%)	
Age (mean in years)	56.57+/-16.65	52.37+/-11.68	0.37
BMI (mean)	24.17+/-3.22	25.48+/-3.95	0.39
BMI in subgroups (%)			
<18 (n=2)	0	2 (1.5%)	0.81
18-25 (n=64)	4 (57.1%)	60 (45.8%)	
>25 (n=72)	3 (42.9%)	69 (52.6%)	
HbA1c (mean)	8.98+/-1.37	8.30+/-1.75	0.31
HbA1C groups			
<7 (n= 26)	0	26 (19.8%)	0.51
7-9 (n=66)	3 (42.9%)	63 (48.1%)	
9-11 (n=30)	3 (42.9%)	27 (20.6%)	
11-13 (n=13)	1 (14.3%)	12 (9.2%)	
>13 (n=3)	0	3 (2.3%)	
Duration of diabetes mellitus in months	83.67+/-77.4	113.29+/-56.74	0.32

Table 14: Diabetic co-morbidities and complications in patients with and without hypomagnesemia

Diabetic comorbidities and complications	Patients with hypomagnesemia (n=7)	Patients without hypomagnesemia (n=131)	P value
Diabetic comorbidities			
Hypertension (n=58)	3 (42.9%)	55 (42%)	0.96
Dyslipidemia (n=107)	7 (100%)	100 (76.3%)	0.14
Diabetic complications			
-Microalbuminuria (n=62)	3 (42.9%)	59 (45%)	0.91
-Nephropathy (n=14)	2 (28.6%)	12 (9.2%)	0.97
-Neuropathy (n=82)	3 (42.9%)	79 (60.3%)	0.35
-Retinopathy (n=24)	3 (42.9%)	21 (16%)	0.07
-Ischemic heart disease (n=18)	2 (28.6%)	16 (12.2%)	0.21
-Cerebrovascular disease (n=2)	0	2 (1.5%)	0.74
-Peripheral vascular disease	0	0	
-Renovascular disease (n=1)	1 (14.3%)	0	

Predictive factors- Drug history

The proportion of patients in each group, who were on sulphonylureas and metformin were comparable. 14% of the hypomagnesemic population and about 6% of the normomagnesemic population were on thiazolidinediones, but the difference was not statistically significant. However, a significant proportion of the hypomagnesemic group was on insulin (85%) as compared to the normomagnesemic group (26%),and that difference was statistically significant with a p value of 0.001.

With regard to the other drugs, which affected the magnesium homeostasis, diuretics were widely used, but the proportion of patients on diuretics were also comparable between the 2 groups, as shown in table 15.

Table 15. Drug list compared between patients with and without hypomagnesemia

Drug	**No of patients with hypomagnesemia(%)**	**No of patients without hypomagnesemia (%)**	**P value**
Diabetic drugs			
Sulphonylureas	5 (71.4%)	87 (66.4%)	0.78
Biguanides	5 (71.4%)	99 (75.6%)	0.80
Thiazolidinediones	1 (14.3%)	8 (6.1%)	0.39
Meglinides	0		
Alphaglucosidase inhibitor	0	1 (0.8%)	
Insulin	6 (85.7%)	34 (26%)	0.001
Other drugs			
Antibiotics			
c. Aminoglycosides	0		
d. Carbenicillin	0		
e. Amphotericin	0		
Diuretics	1 (14.3%)	16 (12.2%)	0.87
Alcohol	0	2 (1.5%)	0.74
Cisplatin	0	0	
Digoxin	0	2 (1.5%)	0.74
Methotrexate	0	0	
Statin	3 (42.9%)	47 (35.9%)	0.71
Theophylline	0	1 (0.8%)	0.82

Comparison of the Mean Magnesium values in different subgroups of patients

The mean magnesium concentration of the total number of 138 diabetics, was **2.06+/-0.23mg/dl.** The table shows the mean serum total Mg concentration in different subsets of patients, like gender, presence or absence of co-morbidities and complications. The mean magnesium concentration was higher in the males than in females (2.1mg/dl vs 2.02mg/dl respectively), and this difference was statistically significant with a p value of 0.04. In different subgroups with regard to presence and absence of co-morbidities and complications, the mean Mg concentration was comparable. Even the mean Mg concentration between the different HbA1c groups were comparable.

Table 16: The mean Mg in different subgroups with and without the complications and comorbidities

Subgroups of Patients	Mean Serum Magnesium	Standard Deviation	P Value
Sex- Male	2.1	0.25	0.044
-Female	2.02	0.2	
HbA1c </= 7	2.05	0.19	NS
7 to 9	2.09	0.2	
9 to 11	2.02	0.31	
11 to 13	2.03	0.27	
> 13	2.02	0.16	
Hypertension- Yes	2.05	0.22	0.42
- No	2.08	0.24	
Dyslipidemia- Yes	2.07	0.24	0.86
- No	2.06	0.2	
Retinopathy- Yes	1.98	0.26	0.08
- No	2.08	0.22	
Microalbuminuria- Yes	2.06	0.23	0.93
- No	2.06	0.23	
Nephropathy - Yes	2.04	0.3	0.75
- No	2.07	0.22	
Neuropathy- Yes	2.08	0.24	0.39
- No	2.05	0.23	
IHD- Yes	2.07	0.34	0.87
- No	2.06	0.21	
CVA- Yes	1.86	0.04	0.23
- No	2.07	0.23	
Renovascular disease-Y	1.7	0	0.12
-N	2.06	0.22	

Drugs and mean magnesium concentration

Table 17 shows the subsets of population with and without the drugs, and their mean magnesium concentration. It was interesting to find a significantly lower mean Mg value among the patients who had been on thiazolidinediones- 1.91 mg/dl, when compared to the patient group who was not on the same-2.07 mg/dl, and the p value was 0.04. With regard to the other drug groups, the mean Mg concentrations were comparable between the 2 groups.

Table 17- The mean Mg concentration in subgroups of different drugs

Drug	**Mean Magnesium**	**Standard Deviation**	**P Value**
Sulphonylurea -yes - no	2.04 2.09	0.22 0.25	0.22
Biguanides -yes - no	2.05 2.08	0.22 0.27	0.69
Thiazolidinediones-yes - no	1.91 2.07	0.28 0.23	0.04
Insulin-yes - no	2.02 2.08	0.3 0.2	0.14
Diuretics -yes - no	2.09 2.05	0.27 0.23	0.57

Analysis of the 24 hour urine magnesium samples:

127 patients among the study patients had given 24 hour urine samples for the estimation of magnesuria. The normal range was taken as 70 to 120 mg per day (76). About 44.8% had features of hypermagnesuria, as shown in Table 18. Renal magnesium handling is very efficient so as to accommodate for the changes in serum magnesium, and hence hypomagnesuria may be relevant only in patients with hypomagnesemia. However, among the patients with hypomagnesemia, inappropriate magnesuria was observed in only 1 patient (14.3%), whereas it was observed in about 46.6% among the normomagnesemic group. This difference was however not statistically significant.

Table 18: 24 hour urine magnesium excretion in relation to serum Mg concentration

24 hours urine magnesium	No of patients with hypomagnesemia (total =7)	No of patients without hypomagnesemia (total = 120)	P value
0-70mg	1 (14.3%)	10 (8.3%)	0.17
71-120 mg	5 (71.4 %)	54 (45%)	
>120 mg	1 (14.3%)	56 (46.7%)	

Diuretics and 24 hour urine magnesium:

It is well known that the urinary Mg excretion is increased, if the patient is on diuretics (28, 84). In table 19, a comparison of the 24 hour urine Mg was done between the patients who had been on diuretics and who had not been on diuretics. 14 patients out of the 127 patients for whom 24 hour urine Mg values were available, had been on diuretics. Out of the 14 patients, 57% had hypermagnesuria, whereas in the patients who had not been on diuretics, 44% had hypermagnesuria, but this difference was also not statistically significant.

Table 19: 24 hour urine magnesium in relation to diuretic use

24 hour urine magnesium	No. of patients on diuretics (total = 14)	No. of patients Not on diuretics (total=113)	P Value
0-70mg	0	12(10.6%)	0.376
71-120mg	6(42.8%)	51(45.1%)	
>120mg	8(57.2%)	50(44.3%)	

Estimation of dietary intake of Magnesium

About 15 patients were asked for a 24 hour recall for diet, and an approximate estimation was made, regarding their intake of daily intake of magnesium from the available information. Most of them were following the dietary instructions as provided by the dieticians of the diabetic endocrine out patient clinic. Their average daily intake was calculated, and the method of estimation is attached as Annexure B. The average daily intake of magnesium in diet of our population is given in table 20.

Table 20: Mean daily dietary consumption of magnesium

	Mean 24 hour diet intake in hypomagnesemic patients (n=7)	Mean 24 hour diet intake in normmagnesemic patients (n=7)	P value
24 hour dietary Mg Intake in mg/day	403	430	0.28

There was no significant difference in dietary magnesium intake between the hypomagnesemic and the normomagnesemic patients.

Estimation of drinking water Magnesium content

Drinking water samples were collected for 10 patients, of which 4 had hypomagnesemia and the other 6 were normomagnesemic.

Ideal drinking water samples should contain atleast 10 mg/dl of magnesium, as evidenced from western literature. It was found that 4 out of the 10 samples, had magnesium content < 10 mg/dl, as shown in table 21.

2 out of the 4 hypomagnesemic patients had low water content of magnesium, which could contribute to their low serum magnesium values. The patients who had low water content of magnesium, were consuming mineral water. The others who had high/ adequate water content of magnesium, had been drinking borewell water. Most of the water supply in Vellore is hard water, which has a high content of magnesium and calcium. Thus the low prevalence of hypomagnesemia in our population, could be attributable partly to the high magnesium content of water. However there was no significant difference between the average Mg content of drinking water between the patients with hypomagnesemia and those with normal serum magnesium values.

Table 21: Magnesium content in drinking water in hypomagnesemic and normomagnesemic groups

	The Mg concentration in the hypomagnesemics (4 patients)	The Mg concentration in the normomagnesemics (6 patients)	P value
	38.7 mg/l (borewell water)	21.7 mg/l (borewell water)	
	1 mg/l (mineral water)	3.6 mg/l	
	21.5 mg/l (borewell water)	28.1 mg/l (borewell water)	
	3.4 mg/l (mineral water)	4.6 mg/l	
		41.4 mg/l (borewell water)	
		41.5 mg/l(borewell water)	
Mean concentration of Mg in drinking water	**16.15 mg/l**	**23.45 mg/l**	0.53

Profile of the hypomagnesemic patients with regard to the factors which could influence the serum Mg values

Table 22 shows the details of the 7 hypomagnesemic patients, including the serum Mg levels, and the other factors which include- quantitative dietary intake of Mg for 24 hours, drinking water content of magnesium, 24 hour urine Mg excretion in mg/day and the drug history- among which diuretic is the most important. The details of drinking water content is available only for 4 of the hypomagnesemic patients. It is obvious that P1- who had a serum Mg level 1.7, had a higher 24 hour urine Mg excretion compared to the other patients, and he had a history of diuretic intake also, which could probably explain the high 24 hour urine Mg excretion. P2 had no obvious explanation for the low serum Mg value. P3 who had the least serum Mg level- 1.2mg/dl, had the lowest value for Mg concentration in the drinking water and 24 hours diet content. P4 had a very low drinking water content of magnesium. The last 3 patients, who had serum values >/=1.58, had a reasonable dietary intake except, a borderline low content in P5, and there was drinking water content information available only for P6, which was adequate. There was no clear cause for the low serum Mg level in P7, as there was no information available on the drinking water content of Mg.

Correlation between hypomagnesemia and hypokalemia- Since hypomagnesemia can also be associated with other electrolyte abnormalities, the serum potassium values were estimated in patients with hypomagnesemia. Of the 5 patients out of the 7 hypomagnesemics, for whom serum potassium values were available, all had normal potassium levels.

Table 22: Hypomagnesemic patients- correlation with regard possible mechanisms for hypomagnesmia

Patient	Serum Mg in mg/dl	Serum K in mmol/l	Diet Mg mg/day	Water Mg In mg/l	24hr urine Mg In mg/day	Diuretic use
P1	1.7	4.8	420	NA	147/2490	Yes
P2	1.46	0	408	38.7	70/810	No
P3	1.2	5.1	348	1	85/1580	No
P4	1.61	3.5	423	3.4	72/3150	No
P5	1.66	3.5	388	NA	111/2360	No
P6	1.58	0	426	21.5	120/1950	No
P7	1.68	4.4	410	NA	99/2050	No

DISCUSSION:

138 patients who were diagnosed to have diabetes mellitus were assessed for hypomagnesemia, and the estimated prevalence was only 5.1% (7/138 patients). The overall prevalence of hypomagnesemia reported in our study, was lower than that reported in other studies of 25% from Delhi, and about 47% from other studies from the West. This study was different from other prevalence studies, in that other factors such as diabetic complications, co-morbidities, drug history, approximate daily intake of magnesium and the magnesium content of drinking water was also documented.

The mean magnesium concentration of our diabetic population was 2.06 +/- 0.23mg/dl, which was higher compared to other populations of diabetics (for example 1.93mg/dl at Bikaner (37), 1.81mg/dl at Chandigarh (38) and 1.44mg/dl at Delhi (39)- from India and from abroad- 1.48mg/dl at Mexico (6)).

In view of the very low prevalence of hypomagnesemia in our study group, tests of statistical significance did not show any major difference, and also multivariate analysis was not possible.

The reasons for lower prevalence of hypomagnesemia in our diabetic population could be the following:

A smaller proportion of patients with poorly controlled diabetes with HbA1c levels >9gm% in our study population. The mean HbA1c of our study population was about 8.3(+/-1.74), as compared to 12.2 (+/- 0.6) % , in the study published by Yajnik et al, who found a significantly lower mean serum Mg level of 2.01 mg/dll compared to 2.1 mg/dl in the non-diabetics (87). Also, Lima et al had found significantly lower Mg content – 1.77+/-0.4 mg/dl, in poorly controlled diabetic group with HbA1c level of

about 9.3+/-2.6% and a prevalence of 47.7% was detected among the diabetic population (27).

Magnesium being a predominantly intracellular cation, serum Mg represents less than 1% of the total body Mg content, so it may not reflect the actual magnesium status of the body. However, most of the studies have used serum total magnesium for total body magnesium estimation. Some studies have found that serum ionized Mg and intracellular Mg may not correlate with serum total Mg content, as suggested by Resnick etal, who found that both ionized Mg and intracellular Mg were lower in diabetics, whereas serum total Mg content was not significantly low. Ionised Mg and intracellular Mg were 0.552+/-0.008mmol/l and 184+/-13.7mmol/l in the diabetics, compared to non diabetics where the values were 0.630+/-0.008mmol/l and 223.3+/-8.3mmol/l respectively. Furthermore, a close relationship was observed between serum ionized Mg and intracellular Mg ($p<0.001$), but not with serum total Mg content(88). However, most of the studies so far have used serum total magnesium, rather than intracellular or ionized magnesium.

The patients were evaluated for probable cause for this low prevalence of hypomagnesemia, including 24 hour dietary intake of Mg and the Mg content of drinking water:

Diet: The approximate dietary intake of Mg over a 24 hour period was assessed for 14 patients, of which 7 were hypomagnesemic and the other 7 were normomagnesemic. The major sources of magnesium in the food supply are dairy products (20%), grain products (20%), meat, poultry and fish (15%), legumes, nuts and soya products (13%) - most of these have been restricted in a diet prescribed for diabetics, which could be

contributing to the higher prevalence of hypomagnesemia in diabetics. The recommended daily intake of Mg is 320-420mg/day. Our population had a mean intake of above 400mg.day, which is much higher than in the studies elsewhere and this could be the explanation for a low prevalence of hypomagnesemia in our diabetic population. The normal diet in our population has been based on unprocessed foods- because most of the population consumes only 5% milled rice, whereas in urban areas 10% milled rice is being consumed. The mean magnesium intake was lower in the group with hypomagnesemia (403mg/day), whereas it was 430mg/day among the normomagnesemic patients, but this difference was not statistically significant. In a study done by Song etal, the median intake of magnesium, in their population was found to be 326mg/day and there was a modest inverse association between magnesium intake and risk of developing diabetes among middle aged women.

Drinking water: The contribution of drinking water Mg to overall Mg intake is about 10% (50). The average daily intake of water in our population was about 1.5-2 litres per day. More than 60% of the patients depend on tap water, which was mainly hard water, which has high Mg content (i.e, >10mg/dl), and most of the normomagnesemic people had a water Mg content of >20mg/dl. This may explain the low prevalence of hypomagnesemia in our population. The average mean water content of magnesium was 16.15mg/l in the hypomagnesemic group and 23.45 mg/dl in the normomagnesemic group, and there are studies to show that upto 40-50% of waterborne Mg may be absorbed compared to about 30% from food (66, 67). It has been documented in various studies that cardiovascular mortality is associated with the degree of hardness of water

(85). Thus waterborne magnesium would have relative importance, particularly in patients whose diets are relatively deficient in magnesium.

The proportions of patients who had been on the different hypoglycemic drugs, was similar in both hypomagnesemics and normomagnesemics, except that a significantly higher proportion of hypomagnesemics were on insulin (85% compared to 26% among the normomagnesemics) and this difference was highly significant (p value - 0.001). However when comparing the actual levels of Mg in patients on insulin (2.02 +/- 0.3 mg/dl) as against those not on insulin (2.08+/- 0.2 mg/dl) there was no significant difference. Yajnik had found that insulin treated diabetics had a lower and non-insulin treated diabetics had higher plasma magnesium concentrations (2.01 mg/dl in the insulin group and 2.28 mg/dl in the non-insulin group, ($p<0.01$) (86). It was also interesting to find that the mean magnesium concentration was significantly lower in the patients on thiazolidinediones- 1.91mg/dl, compared to 2.07mg/dl among the patients not on the same, and the p value was 0.04. This finding was in contrary to the study published by Guerroro, where the diabetics on pioglitazone were found to have a significantly higher serum Mg content, than the diabetics who were not on pioglitazone (1.93 +/- 0.16 mg/dl in the pioglitazone group, whereas it was 1.74 +/- 0.25 mg/dl in the control group, $p < 0.0001$) (87). There is not much of evidence in literature with regard to this association between thiazolidinedione and serum Mg content- it may be that hypomagnesemia is more common in long standing diabetics- in whom thiazolidinediones also may be indicated.

With regard to the drugs which may influence the magnesium metabolism, the proportion of the population who had been on diuretics was comparable between the

hypomagnesemic and the normomagnesemic groups, and the mean Mg concentration among the diuretic users and diuretic nonusers was also comparable, unlike in many other studies where there was a significantly lower mean Mg among the diuretic users (1.87 mg/dl among diuretic users vs. 1.89 mg/dl among the diuretics nonusers (28). And the proportion of patients who had been statins- which are known to be associated with hypomagnesemia, was also similar between the 2 groups. The mean concentration of Mg was similar in the populations who had been on the other drugs.

With regard to baseline characteristics, the study population was comparable to the populations involved in other studies. The mean age was 52.5 +/- 11.9 which was similar to most other studies, for example in Yajnik etal's study it was about 54.7 +/- 1.4 years (86). The mean BMI was 25.4 +/- 3.9 in our study population, which was also similar in Yajnik etal's study (26.7+/-0.5) (86). The duration of diabetes in our study was 85.2 months, which was similar to Lima etal's study, where the mean duration was about 7.3 years (+/-5.4). So our study population was in fact comparable with other study populations, with regard to most of the baseline characteristics, except for a lower mean HbA1c, showing reasonably good overall diabetic control.

Another interesting finding was a significantly higher mean Mg concentration among males than females (2.1 mg/dl and 2.02 mg/dl respectively) with a p value 0.04, which was similar in many other studies, where also there was a higher prevalence of hypomagnesemia in females (40, 86).

In this study, there seemed to be a higher prevalence of dyslipidemia among the hypomagnesemics than normomagnesemics, but the difference was not statistically significant. This higher prevalence of dyslipidemia was probably because dyslipidemia

was defined as LDL>100mg/dl or serum triglycerides >150 mg/dl, which are recommended cut offs for patients with diabetes (3, 10), and were much lower than the cut off taken elsewhere. Similarly prevalence of hypertension was also comparable between the 2 groups.

With regard to diabetic microvascular complications, our study showed no significant association between the proportion of patients in both hypomagnesemics and normomagnesemic, except in retinopathy and nephropathy, where the difference was approaching statistical significance (p = 0.07 and 0.09 respectively). Similarly the mean magnesium concentration was comparable between the groups with and without the microvascular complications, except in retinopathy, where the difference was approaching statistical significance (Mean Mg-1.98 mg/dl and 2.08 mg/dl in patients with and without retinopathy respectively, p=0.08). This was similar to a study by McNair etal, where the serum Mg level among the cohort with diabetes had an inverse correlation with retinopathy (44).

There was no significant difference between the proportion of patients with regard to microalbuminuria, and also the mean Mg concentrations between the groups with and without microalbuminuria was comparable, unlike the results from a study by Corsonello etal, who had shown a significantly lower serum ionized Mg value in patients with microalbuminuria and nephropathy, compared to patients without albuminuria (45), which were 0.86 +/- 0.12 mg/dl, 0.84 +/- 0.09 mg/dl and 1.08 +/- 0.04 mg/dl, with a significant p value <0.001.

Neuropathy both sensory and motor, were also evaluated in the diabetic population and the means and proportions were compared, which showed no statistically

significant difference between the hypomagnesemic and the normomagnesemic groups. This was in contrast to the study by Rodrieguez-Moran and Guerrero- romero, who had proven that hypomagnesemia was present in a higher proportion of patients with foot ulcers, than patients without foot ulcers (93.9% of the 33 patients with diabetic foot ulcers compared with 73.1% of the 66 patients without diabetic foot ulcers; p value= 0.02). Also subjects with foot ulceration had lower serum magnesium levels (1.48 +/- 0.33) than those in the control group (1.68 +/- 0.32), p <0.001 (7).

With regard to macrovascular complications, higher prevalence of coronary artery disease was found in the overall population, but the mean Mg concentration and the proportion of hypomagnesemics were almost similar. The prevalence of cerebrovascular disease and renovascular disease was too low, for any significant difference.

LIMITATIONS

1. The sample size was too small to conduct multivariate analyses in view of the extremely low prevalence of hypomagnesemia in our population.
2. The dietary intake and drinking water magnesium content estimation could be done only in a limited number of patients due to logistical reasons.

CONCLUSIONS

The important conclusions of this study are as follows:

1. The prevalence of hypomagnesemia in our diabetic population was detected to be 5.1% (CI- 1.43%- 8.77%).

2. The mean magnesium concentration in our diabetic population was 2.06+/-0.23 mg/dl.

3. All the 7 hypomagnesemic patients had HbA1c levels above 7 g%

4. The mean HbA1c was higher in the hypomagnesemic group (8.98 g% +/- 1.37), than in the normomagnesemic group (8.30 g% +/- 1.75), though the difference was not statistically significant (p value-0.31).

5. Males had a higher mean magnesium concentration than females (2.1mg/dl in males and 2.02 mg/dl in females), with a p value 0.04.

6. The proportion of patients with retinopathy and neuropathy in the hypomagnesemics was higher than the normomagnesemic population, and the p values were approaching statistical significance (p 0.07 and 0.09 respectively).

7. The difference between the mean magnesium concentration between the patients with and without retinopathy, was also approaching statistical significance (p-0.08).

8. The profile of the microvascular- neuropathy, microalbuminuria and macrovascular complications between the hypomagnesemic and normomagnesemic diabetics were comparable, and there was no statistically significant difference. The mean magnesium concentration was also comparable between the groups with and without each of the micro and macrovascular complications.

9. The proportion of patients who had been on insulin was higher in the hypomagnesemic groups than the normomagnesemic group with a p value of 0.001.

10. The patients who had been on thiazolidinediones had a significantly lower magnesium concentration (1.91 mg/dl) compared to the patients who had not been on thiazolidinediones (2.07 mg/dl), p value was 0.04.

11. 24 hour urine magnesium excretion was almost within normal range among the hypomagnesemic patients.

12. There was no statistically significant difference in the urinary magnesium excretion between the diuretic users and diuretic non-users.

13. There was a significantly higher daily intake of magnesium in our population than in other studies averaging >400mg/day. It was higher in the normomagnesemic group- 430 mg/day, compared to 403 mg/day in the hypomagnesemic group, but the difference was not statistically significant.

14. The magnesium concentration of drinking water is higher in Vellore, in view of the tapwater being hardwater, on which most of our population depends, which could also explain the low prevalence of hypomagnesemia in our diabetic population.

In summary, although hypomagnesemia is a potentially reversible metabolic problem in diabetes, it does not appear to be a significant problem in our diabetic population, when compared to the other studies from India and the west. The causes of this low prevalence could be probably due to the high magnesium content of drinking water, and high daily dietary intake of magnesium in our population. The other factors like the diabetic complications and co-morbidity profile of the hypomagnesemics and normomagnesemic groups were comparable, except for nephropathy and retinopathy which were approaching significance.

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ANNEXURE A: CLINICAL PROFORMA FOR EVALUATION OF PATIENTS

INCLUDED IN STUDY OF HYPOMAGNESEMIA AND ITS ASSOCIATION WITH DIABETES MELLITUS

A. Demographic details: -

1. Name of the patient				2. Date of first visit	
3. Hospital Number		4. Age		5. Sex	M / F
6. Address for communication Mobile number-					

B. Clinical evaluation for Diabetes and its comorbidities: -

Duration of Diabetes Mellitus		Duration of OHA failure		Type of Diabetes mellitus	I / II

COMORBIDITIES	Hypertension	Dyslipidemia

C. Clinical examination- General: -

Weight in Kg		Height in cm		Body mass Index		Waist circumference		Waist hip ratio			
Pulse rate		Blood pressure		/ mm of Hg							

D. Clinical evaluation for Diabetes microvascular and macrovascular complications: - Circle the appropriate response.

NEUROPATHY	Sensory – Monofilament- R- , L- Biothesiometer R- , L-	Motor	Sensorimotor	Foot ulcer
AUTONOMIC NEUROPATHY	Postural drop In Blood Pressure	Impotence		
NEPHROPATHY	Urine microalbumin	24 hour urine protein	Serum creatinine	
RETINOPATHY	Non proliferative retinopathy	Proliferative Diabetic retinopathy	LASER Therapy/ CSME	Vitreous hemorrhage/ Retinal detachment
ISCHEMIC HEART DISEASE	Past Myocardial Infarction/ACS	Stable angina	TMT positivity	PTCA/ CABG
CEREBROVASCULAR DISEASE	TIA	CVA	Carotid stenosis	Bruit/ Doppler abnormality
PERIPHERAL VASCULAR DISEASE	Absent pulses	Claudication pain	Gangrene	Amputation

RENOVASCULAR	Renal bruit	Renal artery stenosis		

E. Drug history- Circle the drugs in use

Sulphonyl ureas	Biguanides	Thiazolidinediones	Meglinides	Alpha glucosidase inhibitors	Insulin

Antibiotics - Aminoglycosides - Carbenicillin - Amphotericin	Diuretics- Acetazolamide f. Furosemide g. Thiazide	Alcohol	Cisplatin
Digoxin	Methotrexate	Statins	Theophyllines
Other drugs- Specify			

F. Investigations: - (Normal values in brackets)

AC / PC (<110/ <140 mg %)		HbA1c (<7)		Serum Sodium		Serum Potassium	
Lipid profile- TC/ TG/ HDL/ LDL		24 hour urine protein/Urine microalbumin		Serum Magnesium		Serum Calcium	
24 hour urine magnesium							

H. Circle the clinical manifestations if present

Palpitations/ dysrhythmias	Weakness	Muscle cramps	Altered mental status	Seizures/ Jitteriness	

L. FINAL DIAGNOSIS

DIABETES MELLITUS CONTROL	GOOD / POOR
-- NEPHROPATHY	Y / N
-- RETINOPATHY	Y / N
-- NEUROPATHY	Y / N
-- ISCHEMIC HEART DISEASE	Y / N
-- CEREBROVASCULAR DISEASE	Y / N
-- PERIPHERAL VASCULAR DISEASE	Y / N
HYPERTENSION	Y / N
DYSLIPIDEMIA	Y / N

ANNEXURE- B: MAGNESIUM CONTENT IN SPECIFIC FOOD ITEMS

(From the book- Nutritive value of Indian foods- Authors- Gopalan C, Ramasanstri B.V, National Institute of Nutrition)

Food stuff	Quantity	Amount	Contents	Magnesium content- mg/100gm of the food item
RICE	1 CUP	200 GMS		Parboiled- 157 5% milled rice- 90 10% milled rice- 64
1DLI	1 LARGE	50 GM	Rice Black gram dal	 130
DOSA	1 number	50 gms	Rice Black gram dal Fat	
CHAPPATHI	1 number	50 gms	Wheat flour Fat	Whole wheat-138 Wheat- vermicelli-42
BREAD	1 slice	20-30 gms		
SAMBAR	½ CUP	100 gms	Tuar dal Veg Fat	90 31- 44
VEGETABLES	½ CUP	100 gms		31-44
MILK	½ CUP	100 ml		33
EGG	1	50gms		5
MEAT	6-8 piees	70 gms		16-25
FISH	1 slice	65 gms		13
CHICKEN		70 gms		25

ANNEXURE C- PATIENT DATA

ANNEXURE C- Patient data 1

excel Hospit						ment	L monofilament	R biothesiometer		Motor	Urine micro
244290a		45	2	48	28.5	4	4	10	12	0	
253867b		49	1	84	26.3	2	2	20	10	0	
954788b		57	2	180	22.8	2	2	18	20	0	
743968c		25	1	12	23.7	2	2	20	20	0	
658301b		48	1	24	31.5	10	4	31	12	0	
953664a		42	2	264	31.2	4	4	14	14	0	
523527a		51	1	168	22.5	2	2	14	16	0	
398162c		64	1	360	30.1	10	4	16	18	1	
963533c		44	2	12	28.3	2	2	19	24	0	
048503a		49	2	60	26.1	2	2	14	10	0	
160369b		74	1	180	25.9	2	2	18	12	0	
844576b		62	2	72	22.2	4	4	18	20	1	
808117b		68	1	144	36.4	10	10	28	34	1	
796537a		42	2	84	29.4	2	2	14	12	1	
017452d		53	1	12	20.9	2	2	6	8	0	
865250b		47	1	84	29.4	2	2	16	9	0	
610233c		68	1	120	22.5	2	2	14	14	0	
940607c		69	2	168	24.7	6	6	80	60	1	
208151d		49	2	48	28.1	2	2	24	11	0	
471150a		50	2	192	24	2	2	16	14	0	
219090d		40	1	4	23	2	2	8	4	0	
389380c		60	2	60	23.1	2	2	13	9	0	
907372c		59	2	48	26.2	4	4	10	10	0	
724779b		68	2	60	24.7	2	2	14	12	0	
	840378	69	2	276	23.9	4	4	3	3	0	
744172c		72	1	240	22.64	10	10	40	40	1	
611653c		61	1	108	20.4	10	10	16	17	1	
071186a		50	2	264	30.8	4	4	14	30	1	
173601d		61	1	60	21	4	4	29	35	0	
	664010	51	2	216	30.4	2	2	16	13	0	
233871d		70	1	36	24.5	4	4	39	43	1	
079028c		58	1	96	21.1	2	2	8	6	0	
380808c		63	2	180	34.1	4	4	17	7	0	
574886c		58	1	300	28.1	4	4	22	32	1	
776861b		52	1	60	24.6	10	10	12	15	1	
617445b		59	2	120	24.8	4	4	13	24	0	
308443b		58	1	84	24.4	4	4	21	30	0	
202469d		22	2	1	18.8	2	2	15	12	0	
141602d		37	2	36	29.1	2	2	20	13	0	
394763a		78	1	60	25.4	4	4	20	20	0	
400011c		58	1	168	22.2	10	10	23	19	1	
643728c		43	1	48	17.9	4	4	23	28	0	
230358d		61	2	36	20.2	4	2	18	11	0	
873300a		56	2	84	27.3	2	2	8	8	1	
300163c		38	2	12	28.1	2	2	12	14	0	
057599D		63	1	36	38.9	2	4	14	16	1	
668766b		40	1	36	26.3	2	2	15	12	0	
709781B		33	2	36	27.1	2	2	14	26	0	
507073b		43	2	3	26.1	2	2	17	15	0	
032624d		35	1	12	25.1	2	2	20	15	0	
089383d		60	1	12	21.5	2	2	21	10	0	
466570c		47	2	12	27	2	2	13	10	0	
017452d		53	2	12	21	2	2	6	8	0	
684048c		60	1	180	25.8	4	4	5	5	0	
105937b		57	1	1	26.8	2	2	12	12	0	
390993b		61	2	132	26.1	2	2	21	17	0	
706096b		43	1	24	25.4	2	2	10	7	0	
647035C		65	1	240	21	10	10	24	29	1	
023537d		61	1	48	23.53	2	2	20	15	0	

s. creatinine	Retinopathy	IHD	CVA	Peripheral vascular disease	Renovascular disease	Sulphonylureas	Biguanides	Thiazolidinediones	Meglinides	Alp inh
0.7	0	0	0	0	0	1	1	0	0	
0.9	0	0	0	0	0	0	1	0	0	
0.8	0	0	0	0	0	0	1	0	0	
1	0	0	0	0	0	0	1	0	0	
1.1	0	0	0	0	0	1	1	0	0	
0.7	0	0	0	0	0	0	1	0	0	
1.4	1	0	0	0	0	1	1	1	0	
0.9	1	0	0	0	0	1	1	0	0	
0.8	0	0	0	0	0	0	1	0	0	
0.8	0	0	0	0	0	1	0	0	0	
1.1	1	1	0	0	1	1	1	0	0	
0.8	0	0	0	0	0	1	0	0	0	
0.8	0	1	0	0	0	0	1	0	0	
0.6	1	0	0	0	0	1	1	0	0	
0.9	0	0	0	0	0	1	1	0	0	
0.9	0	0	0	0	0	1	1	0	0	
1	0	0	0	0	0	1	1	0	0	
0.6	0	0	0	0	0	0	0	0	0	
0.8	0	0	0	0	0	0	1	0	0	
0.9	0	0	0	0	0	1	1	0	0	
1.1	0	0	0	0	0	0	1	0	0	
0.7	0	0	0	0	0	1	1	0	0	
0.9	0	0	0	0	0	1	1	0	0	
0.6	0	0	0	0	0	1	0	0	0	
1	1	1	0	0	0	1	1	0	0	
2.3	1	0	0	0	0	0	0	0	0	
1.2	0	0	0	0	0	1	1	1	0	
1.1	0	0	0	0	0	0	1	0	0	
1	0	0	0	0	0	0	1	0	0	
0.8	0	0	0	0	0	1	1	1	0	
1.1	0	0	0	0	0	0	0	0	0	
0.9	0	0	0	0	0	1	1	0	0	

0.7	0	0	0	0	0	1	1	0	0
1.1	0	1	0	0	0	1	1	0	0
1.1	0	1	0	0	0	0	0	0	0
1.1	0	0	0	0	0	1	1	0	0
1	0	0	0	0	0	1	1	0	0
1	0	0	0	0	0	0	0	0	0
0.7	0	0	0	0	0	0	1	0	0
1.3	0	0	0	0	0	1	0	0	0
1	1	1	0	0	0	1	1	0	0
2	1	0	0	0	0	1	0	0	0
1	0	1	0	0	0	1	1	0	0
0.8	0	0	0	0	0	1	1	0	0
0.9	0	0	0	0	0	0	1	0	0
1	0	0	0	0	0	0	1	0	0
1	0	0	0	0	0	1	1	0	0
0.9	0	0	0	0	0	1	1	0	0
0.8	0	0	0	0	0	1	1	0	0
1	0	0	0	0	0	1	0	0	0
0.9	0	1	0	0	0	0	0	0	0
0.8	0	0	0	0	0	1	1	0	0
0.9	0	0	0	0	0	1	1	0	0
1.2	0	0	0	0	0	1	1	0	0
0.9	0	0	0	0	0	0	1	0	0
0.9	0	0	0	0	0	1	1	0	0
0.8	0	0	0	0	0	1	1	0	0
1	0	0	0	0	0	1	1	0	0
0.8	0	0	0	0	0	0	1	0	0
0.9	0	0	0	0	0	1	1	0	0
1	0	0	0	0	0	1	1	0	0
0.9	1	0	0	0	0	0	1	0	0
1	1	0	0	0	0	1	1	0	0
1	0	0	0	0	0	0	1	0	0
0.7	0	0	0	0	0	1	1	0	0
0.8	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	1	1	0	0
0.8	1	0	0	0	0	0	1	0	0
0.9	1	1	0	0	0	1	1	1	0
1.2	0	1	0	0	0	0	1	0	0
0.9	0	0	0	0	0	0	1	0	0
1	0	0	0	0	0	1	1	0	0
1.2	0	0	0	0	0	1	1	0	0
1	0	0	0	0	0	1	1	0	0
1.2	0	1	0	0	0	1	0	0	0
0.8	0	0	0	0	0	1	0	0	0
0.8	0	0	0	0	0	1	1	1	0
0.8	0	0	0	0	0	1	1	0	0
0.7	0	0	0	0	0	1	1	0	0
1.1	1	0	0	0	0	1	0	1	0
0.9	0	0	0	0	0	0	0	0	0
1.8	0	0	0	0	0	1	1	0	0

0.8	0	0	0	0	0	1	1	0	0
0.7	1	0	0	0	0	1	1	0	0
0.7	0	0	0	0	0	1	1	0	0
0.8	0	0	0	0	0	0	1	0	0
0.9	1	1	0	0	0	1	0	0	0
0.9	0	0	0	0	0	1	1	0	0
1	0	0	1	0	0	1	1	0	0
0.9	0	0	0	0	0	1	1	0	0
0.8	0	0	0	0	0	1	1	1	0
0.8	0	0	0	0	0	1	1	0	0
1.4	0	1	0	0	0	1	0	0	0
0.7	0	0	0	0	0	1	0	0	0
1	0	0	0	0	0	1	1	0	0
1	0	1	0	0	0	0	1	0	0
0.7	0	0	0	0	0	1	1	0	0
0.9	0	0	0	0	0	1	1	0	0
0.7	0	0	0	0	0	0	0	0	0
1.2	0	0	0	0	0	0	0	0	0
1.1	0	0	0	0	0	0	1	0	0
0.6	0	0	0	0	0	1	1	1	0
0.8	0	0	0	0	0	0	0	0	0
0.7	1	1	0	0	0	1	1	0	0
0.7	0	0	0	0	0	1	1	0	0
0.9	0	0	0	0	0	0	0	1	0
0.7	0	0	0	0	0	1	1	0	0
0.6	1	0	0	0	0	1	1	0	0
0.8	0	0	0	0	0	0	0	0	0
0.9	0	0	0	0	0	1	1	0	0
0.9	0	0	0	0	0	1	1	0	0
1.1	0	1	0	0	0	1	1	0	0
1.5	0	0	0	0	0	1	0	0	0
0.8	0	0	0	0	0	0	1	0	0
1.2	0	1	0	0	0	1	0	0	0
3.9	1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	1	1	0	0
0.8	0	0	0	0	0	1	1	0	0
1.5	1	0	0	0	0	0	0	0	0
0.9	0	0	0	0	0	1	1	0	0
1	1	0	0	0	0	1	1	0	0
1.1	0	0	0	0	0	1	1	0	0
0.6	0	0	0	0	0	1	0	0	0
0.9	0	0	0	0	0	0	1	0	0
0.9	0	0	0	0	0	1	1	0	0
2.6	1	0	0	0	0	0	0	0	0
0.7	0	0	0	0	0	0	0	0	0
0.8	1	0	0	0	0	1	1	0	0
0.7	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	1	0	0
0.8	0	0	0	0	0	1	1	0	0
1.1	1	0	0	0	0	1	1	0	0

0.8	0	0	0	0	0	1	0	0	0
0.8	0	0	0	0	0	1	1	0	0
2.1	0	0	0	0	0	1	1	0	0
1	0	0	0	0	0	0	0	0	0
2.1	0	1	0	0	0	1	1	0	0
0.9	1	0	1	0	0	0	1	0	0

Alcohol	cisplatin	Digoxin	Methotrexate	Statin	Theophyllines	HbA1c	Total choles	Triglycerides	HDL	LDL
0	0	0	0	1	0	7.4	186	87	32	
0	0	0	0	1	0	9.7	153	129	33	
0	0	0	0	0	0	6.9				
0	0	0	0	0	0	7.8	154	133	34	
0	0	0	0	0	0	7.2	210	327	38	
0	0	0	0	1	0	8.5	110	112	42	
0	0	0	0	0	0	6.1	203	150	46	
0	0	0	0	0	0	7.8	194	104	39	
0	0	0	0	0	0	7				
0	0	0	0	0	0	7.8	194	107	40	
0	0	0	0	1	0	8.2				
0	0	0	0	0	0	9.6				
0	0	0	0	1	0	5.9				
0	0	0	0	1	0	7.1	237	128	42	
0	0	0	0	0	0	7	128	127	30	
0	0	0	0	1	0	6.9	203	104	41	
1	0	0	0	0	0	6.2	141	105	29	
0	0	0	0	1	0	7.8				
0	0	0	0	0	0	9.6				
0	0	0	0	0	0	7.3	165	104	31	
0	0	0	0	1	0	7.1	226	171	38	
0	0	0	0	0	0	7.4	195	173	41	
0	0	0	0	1	0	7.3				
0	0	0	0	0	0	6.7	245	319	52	
0	0	0	0	1	0	9.4	219	112	53	
0	0	0	0	1	0	7.1	195	195	42	

0	0	0	0	0	0	7.8	163	196	28
0	0	0	0	0	0	6.9	242	223	54
0	0	0	0	0	0	6.8	120	62	28
0	0	0	0	1	0	8.5	269	145	59
0	0	0	0	0	0	11.9	182	87	48
0	0	0	0	0	0	7.1			
0	0	0	0	0	0	9	226	162	45
0	0	0	0	1	0	7.8	124	66	32
0	0	0	0	0	0	7.3			
0	0	0	0	1	0	8.1	188	156	47
0	0	0	0	1	0	11.5	143	180	30
0	0	0	0	0	0	11.3	162	82	39
0	0	0	0	0	0	7.5			
0	0	0	0	1	0	6.6	207	219	36
0	0	0	0	0	0	9.8			
0	0	0	0	0	0	6.6	100	44	36
0	0	0	0	0	0	9	136	74	51
0	0	0	0	0	0	10.2	135	145	30
0	0	0	0	0	0	6.1	183	85	40
0	0	0	0	1	0	7.3	181	134	30
0	0	0	0	0	0	6.9	222	445	50
0	0	0	0	0	0	7.9	166	279	41
0	0	0	0	0	0	9.9			
0	0	0	0	0	0	6.2	164	61	38
0	0	0	0	0	0	7.8	143	172	33
0	0	0	0	0	0	8	136	70	30
0	0	0	0	0	0	7	128	127	30
0	0	0	0	0	0	7.9	176	154	40
0	0	0	0	0	0	11.1	179	138	31
0	0	0	0	1	0	7.6	235	81	41
0	0	0	0	0	0	7	177	323	36
0	0	0	0	0	0	6.3	184	102	37
0	0	0	0	0	0	7	80	40	24
0	0	0	0	0	0	8.3	165	314	34
0	0	0	0	0	0	6.3			
0	0	0	0	1	0	6.8	128	135	34
0	0	0	0	0	0	8.5	103	98	34
0	0	0	0	0	0	12.3			
0	0	0	0	0	0	8.9		93	0
0	0	0	0	0	0	6.3	205	362	35
0	0	0	0	0	0	9.4	155	135	31
0	0	0	0	0	0	13			
0	0	0	0	0	0	9.6	145	180	35
0	0	0	0	1	0	11.7	192	149	33
0	0	0	0	0	0	7.3			
0	0	0	0	0	0	8.6	195	384	34
0	0	0	0	0	0	7.3	151	159	35
0	0	0	0	0	0	11.4		101	
0	0	0	0	1	0	9		166	
0	0	0	0	0	0	11.2		106	
0	0	0	0	1	0	7.5	162	194	37
0	0	0	0	0	0	11.8	207	154	36

0	0	0	0	0	0	6.8	160	125	26
0	0	0	0	0	0	10.1	144	154	37
0	0	0	0	1	0	7.6	133	186	32
0	0	0	0	0	0	7.5	249	342	42
0	0	0	0	0	0	7.3	197	108	41
0	0	0	0	0	0	4.3			
0	0	0	0	1	0	8.7	248	142	52
0	0	0	0	0	0	6.5	176	76	52
0	0	0	0	1	0	11.1	157	73	33
0		0	0	0	0	6.4	190	148	41
0	0	0	0	1	0	7.8	145	191	28
0	0	0	0	0	0	9.7	171	120	37
0	0	0	0	1	0	10.1	137	222	33
0	0	0	0	1	0	9.8	275	121	52
0	0	0	0	1	0	7.8	168	228	36
0	0	0	0	1	0	8	175	73	38
0	0	0	0	1	0	7.7	223	122	46
0	0	0	0	0	0	9.1	171	86	37
0	0	0	0	0	0	7.1	133	228	32
0	0	0	0	0	0	8	199	196	41
0	0	0	0	0	0	7.2		286	
0	0	0	0	0	0	11	170	534	34
0	0	0	0	0	1	9.3	137	166	27
0	0	0	0	0	0	6.1	183	188	39
0	0	0	0	0	0	9.9	165	61	43
0	0	0	0	1	0	6.4	149	109	41
0	0	0	0	1	0	6.1	206	142	41
0	0	0	0	0	0	8.2	188	52	59
0	0	0	0	0	0	10.1			
0	0	0	0	1	0	7	202	174	37
0	0	0	0	0	0	6			
0	0	0	0	1	0	7.7	283	184	61
0	0	0	0	0	0	9	164	129	43
0	0	0	0	1	0	9.7	202	175	33
0	0	0	0	0	0	7.2	132	201	24
0	0	0	0	1	0	12.3	269	105	55
0	0	0	0	0	0	13			
0	0	0	0	0	0	8	233	695	57
0	0	0	0	0	0	8.6	203	148	44
0	0	0	0	1	0	11.7	200	146	41
0	0	0	0	0	0	7	216	97	43
0	0	0	0	0	0	10.3	154	451	41
0	0	0	0	1	0	10.1			
0	0	0	0	1	0	8.6	138	178	35
0	0	0	0	1	0	10.4	223	153	42
0	0	0	0	0	0	7.8	179	146	33
0	0	0	0	0	0	9.2	199	210	35
0	0	0	0	1	0	9.6	171	202	35
0	0	0	0	0	0	8.6			
1	0	0	0	1	0	9.2			
0	0	0	0	1	0	7.3	225	160	46
0	0	0	0	0	0	6.2	192	165	43

0	0	0	0	1	0	10	239	172	48
0	0	0	0	0	0	8.1	182	162	39
0	0	0	0	0	0	8.2	238	79	47
0	0	0	0	0	0	13	208	125	38
0	0	1	0	1	0	7.3	179	126	30
0	0	0	0	1	0	7	186	132	51
0	0	1	0	1	0	7.3	179	126	30
0	0	0	0	1	0	9.8	108	148	33