ORIGINAL RESEARCH

Study of Serum Magnesium in MicrovascularComplications of Type 2 Diabetes Mellitus

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ABSTRACT

Background: Magnesium is a critical ion that is essential for life. It is intimately involved in over 300 enzymatic reactions. It also has important endocrine functions and is required for protein synthesis. Studies have shown that magnesium levels are lower in patients with diabetes compared with non-diabetic controls. Hence this work was undertaken to evaluate the relationship between serum magnesium and diabetes mellitus without and with microvascular complications. Aim: To compare the serum magnesium levels in type 2 diabetes mellitus patients with and without micro vascular complications. Objectives: To estimate serum magnesium levels in type 2 Diabetes mellitus, to correlate serum magnesium level in type 2 diabetics with and without micro vascular complications.

Materials and Methods: Serum magnesium levels of 60 patients having type 2 diabetes with microvascular complications (cases) compared to 60 patients with type 2 diabetes without microvascular complications (control).

Results: Mean duration of diabetes (yrs) was significantly high in cases (3.58) than controls (10.15) ($p<0.001$). FBS and PPBS did not show significant difference between controls and cases. HbA1c (%) was significantly higher among cases (9.93±2.50) than controls (8.98±2.26). Serum magnesium levels were significantly lower among cases (1.46±0.32) than controls (1.92±0.25). Hypomagnesaemia did not significantly differ between various sub groups of micro vascular complications.

Conclusion: Serum magnesium levels were significantly lower in diabetic patients with microvascular complications than in diabetic patients without microvascular complications. Hypomagnesemia was more common in diabetics with poor glycemic control.

Keywords: Serum Magnesium, Hypomagnesemia, Microvacular Complications

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INTRODUCTION

Magnesium is a critical ion that is essential for life. It is intimately involved in over 300 enzymatic reactions. It also has important endocrine functions and is required for protein synthesis.[1] Studies have shown that magnesium levels are lower in patients with diabetes compared with non-diabetic controls.[2]

Diabetes mellitus (DM), characterized by metabolic disorders related to high levels of serum glucose, is probably the most associated disease to Mg depletion in intra and extracellular compartments.[3]

The interrelationships between magnesium and carbohydrate metabolism have regained considerable interest over the last few years. The association between diabetes mellitus and hypomagnesaemia is compelling for its wide-ranging impact on diabetic control and microvascular complications.[4]

Hypomagnesemia has been related as a cause of insulin resistance, also being a consequence of hyperglycemia, and when it is chronic leads to the installation of macro and microvascular complications of diabetes, worsening the deficiency of Mg.[3]

The etiology of hypomagnesaemia cannot be clearly explained and serum magnesium levels have been shown to be inversely related to the severity of diabetes.[4]Hypomagnesaemia is contributed by:

(a) Hyperglycemia which leads to decreased cellular Mg Levels, independent of insulin levels
(b) Osmotic diuresis leads to increased urinary Mg losses and
(c) Concomitant use of diuretics and hypolipidemic agents also increase urinary Mg loss.[5]

Hypomagnesaemia has been linked to poor glycemic control, diabetic nephropathy, retinopathy and diabetic neuropathy.[4]

Magnesium is essential for insulin secretion, insulin receptor interaction, post receptor events (involving tyrosine kinase mediated phosphorylation) and normal carbohydrate utilization (by Mg dependent enzymes).[5] A compromise in these functions leads to insulin resistance in hypomagnesaemia.[5] Oral magnesium supplementation improves insulin sensitivity and metabolic control in type 2 diabetic subjects.[6,7]

Hence this work was undertaken to evaluate the relationship between serum magnesium and diabetes mellitus without and with microvascular complications.

Aims and Objectives of the Study

Aim: To compare the serum magnesium levels in type 2 diabetes mellitus patients with and without micro vascular complications.

Objectives:

1. To estimate serum magnesium levels in type 2 Diabetes mellitus.
2. To correlate serum magnesium level in type 2 diabetics with and without micro vascular complications

MATERIALS & METHODS

Source of Data:

Patients of type 2 diabetes mellitus of age 40-70 years, both outpatient and inpatient in the Department of General Medicine, Kempegowda Institute of Medical Sciences, Bangalore during study period were taken for study considering the inclusion and exclusion criteria.

Methods of collection of data:

1. The diagnosis of type 2 diabetes mellitus was established with the recommended criteria of American diabetes Association.
2. Informed consent was taken from patient and control subjects.
3. A pre-structured and pretested pro forma was used to collect the data.
4. Baseline data including age and sex, detailed medical history including conventional risk factors, clinical examinations and relevant investigations were done.
5. Retinopathy was assessed by Direct Ophthalmoscopy.
6. Nephropathy was determined based on dipstick test for urine microalbumin and renal function tests.
7. Neuropathy was determined by clinical examination and monofilament test.
8. Blood and urine samples were collected for relevant investigations.

9. Serum magnesium was estimated by Calmagite dye method (11) by using auto-analyzer (A25Biosystem). Magnesium reacts with the blue dye,calmagite, in alkaline medium to form red colored complex which is measured at 530-550nm. The intensity of the color formed is directly proportional to the amount of magnesium in the sample. Protein interference and dye precipitation are avoided including the 9-ethylene oxide adduct of pnonylphenol(Bion NE9) and Polyvinyl pyrrolidone (Bion pup). Calcium interference is avoided by preferential combination with EGTA and heavy metal interference is prevented by Potassium cyanide. The reference range for serum magnesium concentration does not vary significantly for age or sex and is closely maintained within a range of 1.7-2.4mg/dl.

Sample size:
Total: 120, 60 cases and 60 controls between age of 40-70years
Cases: 60 patients of type2 diabetes mellitus with micro vascular complications.
Controls: 60 patients of type2 diabetes mellitus without micro vascular complications.

Sampling Method: Purposive sampling.
Type of Study: comparative study.

Inclusion criteria:
Cases: Patients willing to participate in the study, in the age group of 40–70 years with type 2 diabetes with proven micro vascular complications, like nephropathy, neuropathy and retinopathy were selected.
Controls: Patients willing to participate in the study, in the age group of 40–70 years with type 2 diabetes without proven micro vascular complications

Exclusion criteria:
Patients with
1. diabetic keto acidosis
2. coronary artery disease
3. stroke
4. peripheral vascular disease
5. immunological disorders
6. taking magnesium supplements or containing antacids
7. malabsorption syndrome
8. chronic diarrhea
9. chronic renal failure due to factors other than type 2 Diabetes Mellitus
10. pancreatitis
11. alcoholism and
12. liver diseases were excluded from the study

Statistical analysis:
The following methods of statistical analysis have been used in this study.
The results were averaged (mean + standard deviation) for continuous data and number and percentage for dichotomous data are presented in Table and Figure.

RESULTS
The controls and cases were age and sex matched, mean age of controls and cases was 56.82 and 59.07 respectively (p=0.21). Among controls 38 patients were males and 22 patients females, while there were 35 males and 25 females among cases (p=0.57).

There was no statistically significant difference in BMI between controls and cases (p=0.13). Mean duration of diabetes (yrs) was significantly high in cases (3.58) than controls (10.15) (p<0.001). Among cases 46 patients were found to be hypertensive against 21 patients in controls.

Table 1: Comparison of various parameters in controls and cases

	Controls(n=60)	**Cases (n=60)**
Age (years)	56.82±10.07	59.07±9.52
SexMales females	38(63.3%) 22(36.7%)	35(58.3%) 25(41.7%)
BMI	26.06±2.95	27.45±6.60
Durationofdiabetes(yrs)	3.58±3.406	10.15±5.977
Treatment-none OHA Insulin Both	16 38 4 2	5 27 19 9
FBS(mg/dl)	180.30	180.77
PPBS(mg/dl)	255.92	264.90
HbA1c%	8.98±2.26	9.93±2.50
Hypertension	21(35%)	46(76.7%)

Table 2: FBS, PPBS, HbA1c, Serum Magnesium levels

		N	**Mean**	**SD**	**Min.**	**Max.**	**'t' value**	**'p' value**
FBS (mg/dl)	Control (N=60)	60	180.3	84.178	65	524		
	Case (N=60)	60	180.77	67.205	59	414	0.001	0.973
PPBS (mg/dl)	Control (N=60)	60	255.92	109.417	125	564		
	Case (N=60)	60	264.9	94.78	86	566	0.231	0.632
Glycated HbA1c(%)	Control (N=60)	60	8.985	2.2662	6.1	14		
	Case (N=60)	60	9.938	2.5039	6.8	16.3	4.781	0.031
Serum Magnesium	Control (N=60)	60	1.922	0.2512	1.4	2.4		
	Case (N=60)	60	1.468	0.3202	0.9	2.2	74.451	<0.001

FBS and PPBS did not show significant difference between controls and cases. However, HbA1c (%) was significantly higher among cases (9.93±2.50) than controls (8.98±2.26) and serum magnesium levels were significantly lower among cases (1.46±0.32) than controls (1.92±0.25).

Table 3: Distribution of FBS in study population

	FBS (mg/dl)		Total	2 values	p value
	≤130	>130			
Control	20	40	60		
	33.3%	66.7%	100.0%		
Case	13	47	60		
	21.7%	78.3%	100.0%	2.048	0.152
Total	33	87	120		
	27.5%	72.5%	100.0%		

20 patients in controls and 13 patients in cases had FBS below 130mg/dl

Table 4: Glycemic control in study population

	Glycated HbA1c (%)		Total	□ 2 value	„p‟ value
	≤7.0	>7.0			
Control	18	42	60		
	30.0%	70.0%	100.0%		
Case	4	56	60		0.001
	6.7%	93.3%	100.0%	10.909	(S)
Total	22	98	120		
	18.3%	81.7%	100.0%		

Cases had poorer glycemic control compared to controls and it was statistically significant.

Table 5: Distribution of serum magnesium in study population

	Serum Magnesium		Total	□ 2 value	„p‟ value
	≤1.7 mg/dl	>1.7 mg/dl			
Control	13	47	60		
	21.7%	78.3%	100.0%		
Case	51	9	60		<0.001
	85.0%	15.0%	100.0%	48.348	(S)
Total	64	56	120		
	53.3%	46.7%	100.0%		

Hypomagnesaemia was seen more commonly in cases (85%) compared to controls (21.7%) and it was highly significant.
Out of 60 cases, 11 patients (18.3%) had only retinopathy, 8 patients (13.3%) had both retinopathy and nephropathy, 11 patients (18.3%) had retinopathy and neuropathy and 30 patients (50%) had all three complications.

Table 6: Micro vascular complications and serum magnesium

	Serum Magnesium		Total	□ 2 value	„p‟ value
	≤1.7mg/dl	>1.7mg/dl			
OnlyRetinopathy	6	5	11		
	54.5%	45.5%	100.0%		
Retinopathy&Nephropathy	7	1	8		
	87.5%	12.5%	100.0%		
Retinopathy &Neuropathy	10	1	11		

	90.9%	9.1%	100.0%	9.976	0.019
All Three Complications	28	2	30		
	93.3%	6.7%	100.0%		
Total	51	9	60		
	85.0%	15.0%	100.0%		

51 patients out of 60 cases had hypomagnesaemia. Hypomagnesaemia was more common in patients with, ll three complications‟ group, followed by „retinopathy+ neuropathy‟ group, followed by, retinopathy+ nephropathy‟ group, followed by „only retinopathy group, which was statistically significant.

Table 7: FBS, PPBS, HbA1c and serum magnesium in micro vascular complications

		N	**Mean**	**SD**	**Min.**	**Max.**	**„f‟ value**	**„p‟ value**
FBS (mg/dl)	Only Retinopathy	11	156.18	52.911	75	238	0.686	0.564
	Retinopathy& Neuropathy	8	181.50	61.744	100	270		
	Retinopathy& Nephropathy	11	195.73	63.710	86	320		
	AllThree Complications	30	184.10	74.685	59	414		
PPBS (mg/dl)	Only Retinopathy	11	241.09	63.422	134	347	0.758	0.522
	Retinopathy& Neuropathy	8	240.13	81.543	136	385		
	Retinopathy& Nephropathy	11	293.18	112.460	133	566		
	AllThree Complications	30	269.87	101.044	86	551		
Glycated HbA1c(%)	Only Retinopathy	11	9.818	2.6389	7.1	14.4	0.455	0.715
	Retinopathy&Neuropathy	8	10.900	2.4337	6.9	14.0		
	Retinopathy& Nephropathy	11	9.655	1.9521	7.2	12.8		
	AllThree Complications	30	9.830	2.7031	6.8	16.3		
Serum Magnesium	Only Retinopathy	11	1.645	.3934	.9	2.2	2.248	0.093
	Retinopathy& Neuropathy	8	1.563	.2134	1.3	2.0		
	Retinopathy& Nephropathy	11	1.464	.3295	.9	2.2		
	AllThree Complications	30	1.380	.2905	.9	2.2		

However, mean FBS, PPBS, HbA1c and serum magnesium levels did not show significant difference between the different subgroup of complications.

Table 8: serum magnesium and type of treatment.

Group	Serum Magnesium	Treatment				Total	□ 2 value	„p“ value
		OHA	Insulin	Insulin &OHA	No treatment			
Control		9	1	1	2	13		
	≤1.7mg/dl	69.2	7.7%	7.7%	15.4%	100.0		
		%				%		
	>1.7 mg/dl	29	3	1	14	47	1.855	0.603
		61.7 %	6.4%	2.1%	29.8%	100.0 %		
		38	4	2	16	60		
	Total	63.3	6.7%	3.3%	26.7%	100.0		
Case		%				%		
		23	17	8	3	51		
	≤1.7mg/dl	45.1	33.3%	15.7%	5.9%	100.0		
		%				%		
	>1.7 mg/dl	4	2	1	2	9	2.857	0.414
		44.4 %	22.2%	11.1%	22.2%	100.0 %		
		27	19	9	5	60		
	Total	45.0	31.7%	15.0%	8.3%	100.0		
		%				%		

Table 9: serum magnesium and duration of diabetes

Group	Serum Magnesium	Duration Of Diabetes					Total	□ 2 value	„p“ value
		0	1-5 yrs	6-10 yrs	11-15 yrs	>15 yrs			
Control	≤1.7 mg/dl	2	6	5	0	0	13	4.466	0.215
		15.4 %	46.2 %	38.5 %	.0%	.0%	100.0 %		
	>1.7 mg/dl	14	23	7	3	0	47		
		29.8 %	48.9 %	14.9 %	6.4%	.0%	100.0 %		
	Total	16	29	12	3	0	60		
		26.7 %	48.3 %	20.0 %	5.0%	.0%	100.0 %		
Case	≤1.7 mg/dl	1	14	22	8	6	51	3.739	0.443
		2.0%	27.5 %	43.1 %	15.7 %	11.8 %	100.0 %		
	>1.7 mg/dl	1	1	3	2	2	9		
		11.1 %	11.1 %	33.3 %	22.2 %	22.2 %	100.0 %		
	Total	2	15	25	10	8	60		
		3.3%	25.0 %	41.7 %	16.7 %	13.3 %	100.0 %		

There was no significant difference in hypomagnesaemia between various treatment groups or in relation to duration of diabetes mellitus in both controls and cases.

Table 10: correlation of FBS, HbA1c and S.Mg with HbA1c, S.Mg and duration of diabetes

Group			Glycated HbA1c (%)	Serum Magnesium	Duration Of Diabetes
Control	FBS(mg/dl)	Correlation	0.528	-0.253	0.207
		„p“ value	<0.001	0.051	0.112
	GlycatedHbA1c(%)	Correlation		-0.211	0.149
		„p“ value		0.105	0.255
	Serum Magnesium	Correlation			-0.201
		„p“ value			0.123
Case	FBS(mg/dl)	Correlation	0.407	-0.016	0.126
		„p“ value	0.001	0.900	0.339
	GlycatedHbA1c(%)	Correlation		-0.075	0.107
		„p“ value		0.567	0.414
	Serum Magnesium	Correlation			0.075
		„p“ value			0.568

FBS correlates positively with HbA1c and negatively with serum magnesium levels in both controls and cases. Both FBS and HbA1c correlate positively with duration of diabetes and HbA1c correlates negatively with serum magnesium levels in both controls and cases.

DISCUSSION

The present study included 120 type 2 diabetic patients, 60 cases and 60 controls who were age and sex matched. There was no statistically significant difference in BMI between controls and cases (p=0.13).

Mean duration of diabetes (yrs) was significantly high in cases (10.15) than controls (3.58) (p<0.001). Most patients in the control group (48.3%) had duration of diabetes between 1-5 years, while it was between 6-10 years among cases (41.7%). Hypertension was more common among cases (76.7%) than controls (35%).

More patients were on insulin for treatment of diabetes among cases (31.7%) than controls (6.7%). Majority of patients were on oral hypoglycaemic agents among controls (63.3%).

Table 11: comparison of FBS in various studies

	FBS (mg/dl) (controls)	FBS (mg/dl) (cases)
AshaS Khubchandani(2013),[8]	172.00± 15.96	235.44± 18.04
KiranP.Chauhan(2013),[9]	134± 82	161± 130
Presentstudy	180.3±84.17	180.77±67.20

In the present study there was no significant difference between FBS of cases and controls, while in the studies done by Asha S Khubchandani as well as Kiran P. Chauhan it was found that cases had a higher FBS compared to controls.[8,9]

Table 12: comparison of HbA1c in various studies

	HbA1c % (controls)	HbA1c % (cases)
AshaS Khubchandani(2013),[8]	7.80± 0.65	10.60± 1.0
KiranP.Chauhan(2013),[12]	7.8± 4.26	9.2± 4.22

Presentstudy	8.98±2.26	9.93±2.50

In the present study glycemic control as indicated by HbA1c %, was poor among cases than controls, the mean HbA1c of cases (9.93±2.50) was significantly higher than that of the controls (8.98±2.26). The results were similar to the studies done by Asha S Khubchandani,[8] as well as Kiran P. Chauhan,[12] they also found that the meanHbA1c of cases was higher than that of the controls. Previous studies showed that higher level of HbA1C increases risk for development of microangiopathy and macroangiopathy in diabetics. Asha S Khubchandani,[8] in their study,concluded that measurement of HbA1C is not only useful for monitoring of diabetic patients but also provides a conceptual framework for the pathogenesis of secondary sequelae of diabetes.[10]

Table 13: comparison of s.magnesium in various studies

S.Magnesium(mg/dl) »	Controls	Cases
MirzaSharifAhmed Baig(2012),[4]	1.61±0.41	1.29±0.31
AshaS Khubchandani(2013),[8]	1.94± 0.26	1.32± 0.28
Presentstudy	1.922±0.25	1.468±0.32

In the studies done by Mirza Sharif Ahmed Baig,[4] and Asha S Khubchandani,[8] the mean serum magnesium levels in diabetic patients with complications were 1.29±0.31 and 1.32 ± 0.28 respectively. While the mean serum magnesium levels in diabetic patients without complications were 1.61±0.41 and 1.94 ± 0.26 respectively. Both the studies had showed there was significant hypomagnesaemia among diabetic patients with complications than diabetic patients without complications. Mirza Sharif Ahmed Baig,[4] in their study, concluded that the lower levels of serum magnesium may have a bearing on the complication and morbidity in patients of DM, and estimation of serum levels of magnesium may be helpful to monitor the severity of complications in diabetic patients.

Asha S Khubchandani,[8] in their study,suggested hypomagnesemia as a possible risk factor in development and progress of diabetic complications. They also suggested dietary supplementation with magnesium in addition to classical therapies for diabetes may help in prevention or delaying of diabetic complication.

The results in present study also were similar and showed that the mean serum magnesium levels were significantly lower among cases (1.468±0.32), that is diabetic patients with micro vascular complications than in controls (1.922±0.25), that is diabetic patients without micro vascular complications.

Studies have shown that although the binding of insulin to its receptor does not appear to be altered by magnesium status, the ability of insulin once bound to receptor to activate tyrosine kinase is reduced in hypomagnesaemia states.

As a result reduced peripheral glucose uptake and oxidation are often noted in subjects with hypomagnesaemia. Thus hypomagnesemia may be a possible risk factor in development and progress of diabetic complications.

The precise mechanism for development of microvascular changes is not fully understood, it is possible that hypomagnesaemia inhibits prostacyclin receptor function producing an imbalance betwee prostacyclin and thromboxane effect which has marked atherogenic potential which is responsible for microvascular complications.

In the present study mean FBS, PPBS, HbA1c and serum magnesium levels did not show significant difference between the different subgroup of complications.

In the study done by S.S.Antin,[12] hypomagnesaemia was more common in „Only Retinopathy group followed by „Retinopathy+ Neuropathy" group, followed by

„Retinopathy+ Nephropathy‟ group. The study did not have any patients with „All three complications‟.

However in the present study it was found that hypomagnesaemia was more common in patients with „All three complications‟ group, followed by „Retinopathy+ Neuropathy‟ group, followed by „Retinopathy+ Nephropathy‟ group, followed by „Only Retinopathy group, which was statistically significant.[10,11]

In the present study there was no significant difference in hypomagnesaemia between various treatment groups. There was also no significant difference in hypomagnesaemia in relation to duration of diabetes, similar to the study done by S.S.Antin.[12]

In the present study FBS and HbA1c correlate negatively with serum magnesium levels in both controls and cases. FBS correlates positively with HbA1c, similar results were found in the study done by Asha S Khubchandani.[8]

Some studies have shown that oral supplementation with MgCl2 solution restores serum magnesium levels; improving insulin sensitivity and metabolic control in type 2 diabetic patients with decreased serum magnesium levels. However, in the present study magnesium supplementation and its effects towards magnesium levels or metabolic control was not done, which can be taken as a limitation.

CONCLUSION

1. Serum magnesium levels were significantly lower in diabetic patients with microvascular complications than in diabetic patients without microvascular complications.
2. Hypomagnesaemia was more common in diabetics with poor glycemic control.
3. Hypomagnesaemia did not significantly differ between various sub groups of microvascular complications.

Hypomagnesaemia may be a factor in type 2 diabetes mellitus patients leading to various complications.

Hence, it is important to regularly monitor magnesium levels in all type 2 diabetic patients.

Further studies on the role of magnesium supplementation in type 2 diabetes mellitus patients are necessary.

Acknowledgment

The author is thankful to Department of General Medicine for providing all the facilities to carry out this work.

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