

Research Article**Estimation of total and ionized magnesium and its correlation with the glycated hemoglobin in newly diagnosed subjects of type 2 Diabetes Mellitus**Madaan H¹, Agarwal P², Reddy VS¹, Garg R¹, Gupta K³, Panjeta P¹, Kulshreshtha M*⁴¹Department of Biochemistry, ²MBBS (IIRd Prof), ³Department of Ophthalmology, BPS Government Medical College for women, Khanpur Kalan, Sonapat, Haryana,⁴Modern Institute of Medical Sciences, Indore, India***Corresponding author**

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Abstract: Mg deficiency underlies the mechanism of insulin resistance of conditions associated with altered glucose tolerance, such as t2DM. Despite of many reports showing deranged magnesium level in t2DM, most of these studies have focused on level of t Mg in spite of the fact that iMg is the physiological form. Furthermore, data on iMg in newly diagnosed t2DM yet to start treatment is scarce. Therefore, this study was undertaken to evaluate both tMg and iMg level in newly diagnosed type 2 diabetes mellitus. This study was conducted to evaluate serum levels of tMg and iMg in 30 patients with newly diagnosed t2DM and 30 non-diabetic healthy controls, recruited from the out-patient department of Medicine, BPS Government Medical College for Women, KhanpurKalan, Sonapat, and Haryana. Fasting venous blood was drawn from each participant for the biochemical analysis of FBS, HbA1C, tMg and iMg. The results are Compared to controls, the FBS, HbA1C and tMg levels were significantly increased (1.17 ± 0.50 vs. 0.86 ± 0.10 ; $p=0.002$), whereas the iMg level was significantly decreased (0.52 ± 0.04 vs. 0.55 ± 0.06 ; $p=0.02$) in type 2 diabetic patients. The findings of significant association in non-diabetic control group ($r=0.558$, $p=0.001$) and no significant association between tMg and iMg in the patient group ($r=0.245$, $p=0.192$) strengthens the concept of altered Mg metabolism in diabetes. There were no significant associations of FBS and HbA1C with tMg and iMg in the diabetic patient group. In conclusion the observation of decreased physiologically active form of Mg (iMg), despite increased total Mg and loss of correlation between tMg and iMg, suggests that Mg metabolism in diabetes is significantly altered though without any relation to glycemic control.

Keywords: Type 2 DM, Serum Total Mg, Serum Ionized Mg.

INTRODUCTION

Diabetes mellitus (DM) is one of the leading causes of death, worldwide, with increased prevalence during the past 20 years [1]. India has been described as a diabetic capital of the world with about 30 million diabetic people. This increase in diabetes is attributable to a rise in new cases of type 2 diabetes mellitus [2]. Magnesium (Mg) is one of the most abundant and important inorganic bio-cation involved in several biological processes. In circulation, out of the total Mg (tMG), one third of Mg is in bound form to proteins leaving the other two thirds biologically available in the form of free and ionized magnesium (iMg) [3]. Magnesium deficiency is multifactorial and is common in 7-11% of hospitalized patients. Mg deficit is categorized as Mg deficiency due to altered Mg intake and Mg depletion due to dys regulation of factors controlling magnesium status [4].

Altered magnesium metabolism has been described in clinical conditions of insulin resistance,

including metabolic syndrome, hypertension, altered glucose tolerance, aging, and t2DM. Mg deficiency underlies the mechanism of insulin resistance in conditions associated with altered glucose tolerance, such as t2DM [5]. Epidemiological and multi-centric studies have demonstrated an inverse relationship between the ingestion of food rich in Mg and the risk of diabetes [6]. There are cohort studies showing an inverse association between the Mg intake and the risk of t2DM [7, 8]. Positive correlation between insulin sensitivity and the Mg intake has also been reported [9, 10]. In contrast, there are also studies showing no relationship between Mg intake and t2DM risk. Previous study by Mikhail et al. does not support routine Mg supplementation or monitoring in t2DM [11].

Corsonello *et al.*; reported a significant negative association between iMg and glycated hemoglobin (HbA1C) in t2DM patients. They further concluded that micro albuminuria and clinical proteinuria, as well as poor glycometabolic control and

hypertriglyceridemia, are associated to alterations in magnesium metabolism [12]. In another study from China investigating serum and urine Mg levels across pre-diabetes or diabetes with and without complications, serum Mg levels were significantly lowered in impaired glucose tolerance, impaired fasting glucose, type 1 and type 2 DM patients when compared to control subjects. The urinary Mg levels were significantly increased only in t1DM and t2DM patients compared to controls. It was also showed that Simvastatin treatment in t2DM patients selectively reduced serum calcium levels and urinary Mg levels [13].

In a study from Nigeria, Nsonwu *et al.*; studied serum and urine Mg levels in diabetic and non-diabetic groups. They found no significant difference in serum Mg level between groups ($p > 0.05$). Despite of significantly increased levels of urinary Mg in diabetic patients versus non-diabetic controls, they found no significant differences in the serum and urine Mg levels ($p > 0.05$) in diabetics with poor glycemic control (HbA1c $> 8.0\%$) and those with good glycemic control (HbA1c $< 8.0\%$). There was a significant negative correlation between serum Mg and urine Mg levels, suggesting that diabetes alters the metabolism Mg by increasing urinary excretion and lowering serum levels [14] in another study by Ajibola *et al.* no significant difference has been observed in magnesium levels between diabetic t2DM patients and controls [15].

Similar to a study by Nsonwu *et al.*; [14] in a study from Bangladesh by Ferdousi *et al.*; [16] a significant decrease in Mg and Zinc in t2DM patients when compared to non-diabetic controls was reported. A cross sectional study from Sudan reported a significant reduction in the plasma levels of Mg in the diabetic group when compared to that of control group. A significant negative correlation found in that study between Mg and HbA1C suggests association of hypo magnesemia with disease severity [17].

Indians possess high ethnic susceptibility for developing diabetes early at young age. A recent study from India demonstrated a significant decrease in serum Mg level in patients with diabetes and diabetes associated complications when compared to non-diabetic controls. In this study, there were also significant associations between HbA1C and serum Mg [18]. Maula *et al.*; studied serum Mg level in 50 t2DM patients and 50 non-diabetic controls and found that serum Mg level in t2DM patients were significantly decreased than that in healthy controls [19]. In a case-control study involving 100 diabetic cases and 100 non-diabetic controls, Mohanty *et al.* reported significantly decreased Mg and a significant negative correlation between Mg and HbA1C in diabetic patients [20]. Antin *et al.*; reported 35% prevalence of hypo magnesemia among diabetes patients and they also reported significantly increased

prevalence in diabetics with micro vascular complications compared to diabetics with no micro vascular complications [21]. Mishra *et al.*; reported that, the serum Mg levels were significantly lowered in a group of 45 diabetic patients when compared to a control group comprising of 25 non-diabetic healthy controls. They observed a significant negative association between serum Mg and FBS and duration of the disease [22] another study, serum Mg levels were reported to be decreased with a rise in HbA1c levels and duration of t2DM [23].

Despite Mg being described as a forgotten ion until recently, there has been enormous research interest worldwide. Despite of many reports showing deranged magnesium level in t2DM, most of these studies have focused on level of tMg in spite of the fact that iMg is the physiological form [24]. Many drugs given to diabetic patients too may affect the Mg metabolism and levels. The data on iMg in newly diagnosed t2DM is scarce. Therefore, this research was undertaken to study the initial status of both tMg and iMg level in newly diagnosed type 2 diabetes mellitus.

MATERIAL AND METHODS

The study recruited 30 (15 males and 15 females) newly diagnosed patients with t2DM from the outpatient department of Medicine, at BPS Government Medical College for Women, Khanpur Kalan, Sonapat, Haryana from July to September 2014. Thirty healthy non-diabetic participants (15 males and 15 females) were recruited as control group. Diabetes was diagnosed according to American Diabetes Association (ADA) (fasting blood sugar > 126 mg/dL or HbA1C $> 6.5\%$). Persons who were on regular treatment for diagnosed diabetes, diuretic, laxative, recent history of fluid infusion, smokers, alcoholics, subjects with cancer, hypertension, renal and hepatic diseases, any other acute/chronic illness, pregnant, postmenopausal women were excluded. The study was approved by the institutional ethical committee and informed consent was obtained from the participants.

Morning fasting venous sample was collected for HbA1c, blood glucose, total and ionized magnesium estimation. Plasma levels of fasting glucose were measured by enzymatic glucose-oxidase-peroxidase method on Roche/Hitachi Modular P-800 analyzer. The HbA1c level was measured by immuno turbidimetry method using tetra decyl trimethyl ammonium bromide (TTAB method) using commercial kits from Roche diagnostics, on Roche/Hitachi Modular P-800 analyzer. Total magnesium was analyzed on Roche/Hitachi Modular P-800 analyzer, using Xylitol blue method. Ionized magnesium was analyzed by ISE (Ion Selection Electrode) using Nova Biomedica Phox ultra. All the data obtained were present as Mean \pm SD. Differences in variables between groups were tested using two-tailed, unpaired student t-test/Independent sample test. Pearson/Spearman correlation analysis was

employed to study the associations between variables in both control and patient groups. All statistical analyses were conducted using the SPSS for Windows; version 20.

OBSERVATIONS AND RESULTS

The cases (15 males and 15 females, mean age 58.0 ± 12.7 years) and controls (15 males and 15 females, mean age 46.3 ± 14.0 years) were age and sex matched. The Mean and SD of FBS, HbA1C, tMg and iMg in patient and control groups are shown in Table 1. Compared to controls, there was a significant increase in FBS in the patient group (95.06 ± 10.97 vs. 197.40 ± 87.94 mg/dL; $p < 0.0001$). There was a significant increase in the level of HbA1C in patient group when compared to controls ($5.32 \pm 0.55\%$; $p < 0.0001$). Serum levels of tMg were increased significantly in the patient group when compared with the control group (1.17 ± 0.50 vs. 0.86 ± 0.10 ; $p = 0.002$, Fig 1). It was observed that the iMg level was significantly decreased in the patient group when compared with the control group (0.52 ± 0.04 vs. 0.55 ± 0.06 ; $p = 0.02$, fig 2).

The study variables were tested for any differences between male and female patients in the diabetic group (table 2). The FBS was found to be increased in female patients when compared to male patients, but the statistical significance of this difference was marginal (228.60 ± 63.84 vs. 166.20 ± 99.29 mg/dL; $p = 0.05$). There was a significant increase in HbA1C among female patients when compared to their male counterparts (10.69 ± 2.41 vs. $8.49 \pm 1.99\%$; $p = 0.01$). Despite these significant changes, there were no significant changes in tMg and iMg between male and female patients.

While in the control group, there was a significant positive correlation between tMg and iMg ($r = 0.558$, $p = 0.001$), there was no significant correlation between tMg and iMg levels in the diabetic patient group ($r = 0.245$, $p = 0.192$). In the patient group, the only significant association found was between FBS and HbA1C ($r = 0.840$, $p < 0.0001$). There were no significant associations of FBS and HbA1C with tMg and iMg observed in the patient group.

Table 1: the Mean \pm SD values of study variables between control and patient groups.

Variable	GROUP	N	Mean	Std. Deviation	P value
FBS(mg/dL)	Controls	30	95.0667	10.97625	<0.0001
	Patients	30	197.4000	87.94536	
HbA1C(%)	Controls	30	5.3233	.55316	<0.0001
	Patients	30	9.5933	2.44511	
tMg(mg/dL)	Controls	30	.8610	.10327	0.002
	Patients	30	1.1723	.50324	
iMg(mmol/L)	Controls	30	.5580	.06283	0.02
	Patients	30	.5270	.04129	

FBS: fasting blood sugar, HbA1C: glycated hemoglobin, tMg: total magnesium, iMg: ionized magnesium. (A p value of <0.05 has been considered to be statistically significant)

Table 2: The Mean \pm SD values of study variables between male and female diabetic patients.

Variable	GROUP	N	Mean	Std. Deviation	P value
FBS(mg/dL)	Female	15	228.60	63.84	0.050
	Male	15	166.20	99.29	
HbA1C(%)	Female	15	10.69	2.41	0.011
	Male	15	8.49	1.99	
tMg(mg/dL)	Female	15	1.03	0.44	0.141
	Male	15	1.30	0.53	
iMg(mmol/L)	Female	15	0.53	0.049	0.462
	Male	15	0.52	0.032	

FBS: fasting blood sugar, HbA1C: glycated hemoglobin, tMg: total magnesium, iMg: ionized magnesium. (A p value of <0.05 has been considered to be statistically significant)

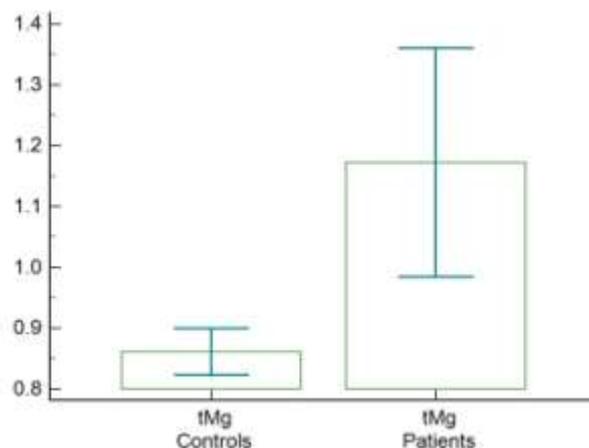


Fig 1: Changes in total-Magnesium (tMg; mg/dL) level between control (n=30) and type2 diabetes mellitus patient groups (n=30).

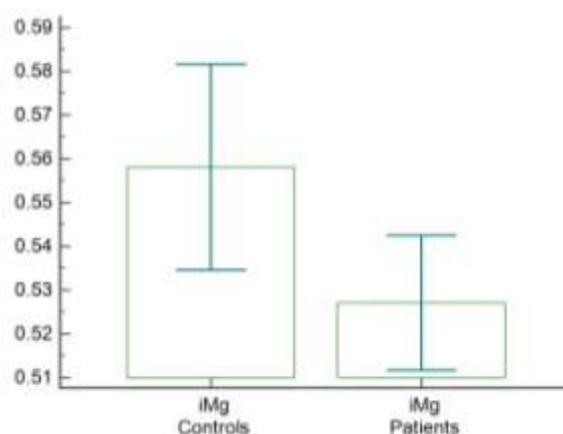


Fig 2: Changes in ionized-Magnesium (iMg; mmol/L) level between control (n=30) and type2 diabetes mellitus patient groups (n=30).

DISCUSSION

It is evident from our results that Mg metabolism is deranged in t2DM as tMg levels were increased and iMg levels were decreased in the diabetic patients along with a loss of correlation between tMg and iMg values as compared to the control group. This suggests a deficit in the physiologically active Mg despite increased total Mg. Female diabetics had higher FBS and HBA1C reflecting poorer glycemic status, but did not have any significant difference in iMg or tMg levels as compared to male diabetics. Further there were no correlations between tMg or iMg with FBS or HBA1C.

Hypo magnesemia has been defined as serum i-Mg <0.46 mmol/L. [25]. In the present study, although we report a decrease in iMg level in diabetic patients, this decreased value (Mean iMg 0.52 mmol/L) is well above the diagnostic cutoff of hypo magnesemia. However, our finding of a significant difference in iMg between diabetic patients and controls could probably suggest future risk of hypo magnesemia in our diabetic patient group.

Our findings are well in line with the previous studies in support of Mg deficiency in t2DM [12, 25]. Deficiency of Mg has been described as a consequence of hyperglycemia and cause for insulin resistance in diabetes and is further implicated in macro vascular and micro vascular complications of diabetes [5].

Magnesium is directly involved in numerous important biochemical reactions, and particularly is a necessary cofactor in over 300 enzymatic reactions and specifically in all those processes that involve the utilization and transfer of adenosine triphosphate (ATP). Thus, intracellular Mg is a critical cofactor for several enzymes in carbohydrate metabolism, and because of its role as part of the activated Mg-ATP complex required for all of the rate-limiting enzymes of glycolysis, regulates the activity of all enzymes involved in phosphorylation reactions. Significant decrease in these enzyme activities were previously reported in type 2 diabetic disease [26]. Magnesium deficiency may result in disorders of tyrosine-kinase activity on the insulin receptor, an event related to the development of insulin resistance and decreased cellular glucose utilization. Therefore, it is

clear that decreased iMg status indicates decreased insulin sensitivity in t2DM [27]. It has been also showed that insulin action strictly depends on cellular iMg levels [28]. A cell with lower iMg has been reported to be less responsive to glucose [29]. The mechanisms of Mg deficiency in diabetes have not been completely described. Insulin resistance and urinary excretion, dietary deficiency, reduced absorption of Mg are important factors in diabetes [11,30]. There is also evidence indicating role of calcium in addition to Mg in insulin action and diabetes. Paolisso and Barbagallo [28] suggested that the low availability of intracellular Mg diminishes the tyrosine kinase activity and increases the vascular constriction mediated by calcium, hindering the relaxation of cardiac and smooth muscles; and in this way, interfering in the usage of the cellular glucose. These mechanisms contribute to raise the blood pressure, peripheral insulin resistance and type 2 diabetes.

Circulating Mg exists in three forms: a protein-bound fraction (25% bound to albumin and 8% bound to globulins), a chelated fraction (12%), and the physiologically active ionized fraction (55%). Because Mg is predominantly an intracellular ion, and in the serum only the ionized active form is metabolically available, its total serum concentrations may not reflect the Mg status [29-31]. In our study, the non-diabetic control group had high levels of both tMg and iMg than in diabetic patient group. There was also a significant positive correlation between tMg and iMg (Spearman's rho correlation coefficient=0.558, p=0.001) in the control group suggesting normal Mg metabolism in non-diabetic state. However, in the diabetic patient group, despite of increase in tMg levels, it was found that iMg status in significantly decreased when compared to control group. This clearly suggests a significant alteration in Mg metabolism leading to low levels of biologically active form of Mg, iMg. Furthermore, there was no significant association between tMg and iMg in the patient group (Spearman's rho correlation coefficient=0.245, p=0.192). This finding strongly support that Mg metabolism is altered leading to decreased iMg levels in diabetes. In the diabetic group, despite of a significant positive association between FBS and HbA1C (Spearman's rho correlation coefficient=0.840, p<0.0001), there were no significant associations of FBS and HbA1C with serum tMg and iMg levels. This observation might be due to less sample size of the study and the newly diagnosed nature of diabetes. Limitations of this study: In addition to less sample size, lack of data on serum calcium levels and urinary Mg status are shortcomings of this study.

CONCLUSION

Serum levels of Mg are altered in patients with type 2 diabetes mellitus. In this study, it is evident that the physiologically active form of serum Mg, ionized Mg is significantly lowered in type 2 diabetes than in non-diabetic controls, despite higher total Mg

levels. The findings of significant association in non-diabetic control group and no significant association between tMg and iMg in the patient group strengthen the concept of disturbed Mg metabolism in diabetes. In addition to increased tMg level, the decrease in iMg is well above the reference level, not enough to be called as hypo magnesemia. Furthermore, there were no significant associations of FBS and HbA1C with tMg and iMg in the diabetic patient group, which might be due to less sample size of the study. This study demonstrated the need to further investigate the levels of tMg and iMg separately along with Mg metabolism and its implications in type 2 diabetes mellitus.

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