HYPOMAGNESEMIA IN DIABETIC PATIENTS: CORRELATION WITH OXIDATIVE STRESS

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ABSTRACT

Magnesium plays an indispensable role in glucose homeostasis. Hyperglycemia and insulin on the other hand, affect plasma and erythrocyte magnesium differently. The present study was carried out to determine whether magnesium deficiency exists in diabetic patients and whether it has some correlation with oxidative stress and duration of disease. To carry out the study, serum RBC magnesium, blood glucose, plasma insulin, antioxidants (vitamins E and C, uric acid, and thiols) and MDA (malondialdehyde) were measured in the diabetic patients having different duration of disease and compared with normal subjects of the same age group. Strong positive correlation between serum RBC magnesium and TRAPc, a measure of total antioxidant parameters ($r=0.66$, $p<0.005$) was observed in these patients. Significant negative correlation was also observed between serum RBC magnesium and blood glucose ($r=-0.388$, $-p<0.05$). These results suggest that hypomagnesemia observed in diabetic patients might play a role in regulating the antioxidant potential in both diabetic patients and normal subjects, as pathophysiology of magnesium deficiency has been independently related to a state of increased oxidative stress. Therefore it may be hypothesized that hypomagnesemia may accelerate the process of oxidative stress in diabetes.

KEY WORDS : Diabetes; Hypomagnesemia; Oxidative stress

INTRODUCTION

Chronic and persistent hyperglycemia has been well established as an important factor in late diabetic complications (1,2). The mechanism by which hyperglycemia causes these complications appears to be multifactorial. Apart from the various known factors, a growing number of recent evidences indicate that magnesium deficiency may play a novel role in the development of diabetic complications (3,4).

Magnesium, the second most abundant intracellular cation has a fundamental role in carbohydrate metabolism in general and in the action of insulin in particular. A complex interplay exists between magnesium and glucose metabolism. Magnesium is an essential cofactor required for the generation of both aerobic and anaerobic energy from carbohydrate metabolism (5). Resnick et al (6) have formulated an ionic hypothesis and postulated that altered intracellular steady state concentrations of ions, such as magnesium, act as a final common pathway to regulate cellular metabolism in general and cellular glucose homeostasis, insulin sensitivity, and blood pressure in particular. Various clinical variables may modify the status of magnesium in the diabetic patients which include the type of diabetic disease, duration, the severity of impairment of glucose metabolism, presence of obesity, kind of therapy practiced and renal function. Hypomagnesemia has been correlated with both impaired glucose tolerance and insulin resistance in non-diabetic elderly patients (7). Although in diabetes poor glycemic control is associated with magnesium deficiency, hypomagnesemia is not corrected by improvement in the metabolic control as documented by glycosylated hemoglobin (8).

Magnesium depletion has been suggested to be related to the development of diabetic microvascular disorders and retinopathy (9), pathogenesis of hypertension (10), atherosclerosis (11) and cardiac arrhythmias (12). Magnesium deficiency has been suggested to be a state of increased oxidative stress as documented by recent studies (13,14). Magnesium itself has been reported to posses antioxidant properties (15,16).

Based on these observations, it seemed interesting to evaluate the concentration of serum RBC magnesium in type 2 diabetic patients, in an attempt to point out the significance of magnesium status in diabetes. To correlate the magnesium depletion with altered antioxidant potential and other biochemical changes observed in diabetes, we measured the major antioxidants present in plasma and calculated TRAP as proposed by Wayner et al (17) and made an attempt to correlate it with hypomagnesemia.

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MATERIAL AND METHODS

100 diabetic patients on restricted diet plus oral hypoglycemic drug therapy on regular follow-up at Department of Endocrinology, PGIMER Chandigarh were selected for the study (60 males and 40 females, ranging in age from 30 to 65 years). The duration of diabetes ranged between newly diagnosed to 20 years. None of the selected patients were taking hypolipidemic drugs or were on antioxidant supplementation. The patients were subdivided into five sub-groups depending upon the duration of diabetes (recently diagnosed, five, ten, fifteen and twenty years of duration). Informed consent was obtained from the patients to participate in the study and the results were compared with normal healthy subjects. The age group of the normal subjects was comparable to that of the diabetic patients.

Biochemical Analysis

Venous blood sample from patients and normal subjects was drawn in the morning after an overnight fast under standardized conditions and divided into three parts (in the heparinized tubes for RBC magnesium and GSH, in oxalate-fluoride tube for glucose and clotted blood for other parameters). Serum was separated within one hour of collection. Blood glucose, serum uric acid, cholesterol, triglycerides and HDL-cholesterol were measured by standard enzymatic reagent kits. LDL and VLDL were calculated by Friedwald formula (18). Magnesium was estimated colorimetrically by dye method (methyl thymol blue) (19). Serum MDA, vitamin E, vitamin C, thiols and RBC GSH were measured by the methods of Beuge and Aust (20), Marthinek (21), Roe and Kuether (22), Koster et al (23) and Beutler et al (24) respectively. TRAPc was calculated as per the formula proposed by Wayner et al (17).

Statistical Analysis

Statistical analysis was done by comparing diabetic patients with normal subjects using Student 't' test. Pearson correlation coefficient was performed to determine the way in which the variable of interest (magnesium-dependent variable) was influenced by several other variables (predictor variables).

RESULTS

The laboratory findings of all the patients are summarized in tables 1,2 and 3. The mean serum magnesium (1.81±0.12 mg/dl vs.2.06±0.068 mg/dl) and RBC magnesium (4.75±0.22 mg/dl vs. 5.2±0.22 mg/dl) levels in all the diabetic patients were found to be significantly reduced as compared to healthy subjects ($p<0.02$). It was shown that in each sub-group, diabetics had lower serum magnesium levels as compared to controls. Overall, 25% of the diabetic patients had significant hypomagnesemia having serum magnesium level less than 1.7 mg/dl. The levels of fasting serum insulin were significantly higher in the diabetic patients as compared to non-diabetic controls ($p<0.001$) (Table 1).

The levels of plasma vitamin C, vitamin E, thiol group and uric acid were significantly reduced in the

Table 1 : Plasma Glucose, Magnesium, Insulin and RBC Magnesium in Diabetic Patients.

Parameters	Normal Subjects	Recently Diagnosed	5 years	10 years	15 years	20 years
Plasma Glucose (mg/dl)	91.5±5.87	182.5±35.90*	185±40.86*	170.05±41.87*	176.3±27.5*	173.55±25.27*
Serum Mg (mg/dl)	2.06±0.068	1.95±0.089*	1.87±0.057*	1.82±0.04*	1.77±0.04*	1.65±0.10*
RBC Mg (mg/dl)	5.2±0.15	4.98±0.08*	4.93±0.08*	4.74±0.14*	4.63±0.13*	4.48±0.13*
Insulin (µu/ml)	15.9±6.28	34.3±7.83*	31.2±8.21*	41±14.81*	39.5±8.62*	41.4±11.43

*All values are mean ± SD. Total number of patients was 20 in each group. p value <0.05 was considered to be significant. (*p<0.001, **p<0.01)*

diabetic patients as compared to the healthy subjects. Serum MDA levels-the marker of lipid peroxidation, was significantly elevated in the diabetic patients than control subjects (Table 2). Dyslipidemia was also observed in the diabetic patients as compared to the control groups as shown by moderate elevation of serum cholesterol, triglyceride, LDL and VLDL levels and decrease in the HDL-cholesterol levels (Table 3).

significant for each sub-group individually. The correlation with glucose, TRAPc, and MDA is shown in respective figures (Fig. 1,2,3). In our study magnesium correlated positively with vitamin C, vitamin E, RBC GSH, thiols, uric acid and negatively with glucose, insulin and MDA (Table 4). Both serum triglycerides and cholesterol levels tended to correlate inversely with serum magnesium. However the

Table 2 : Levels of Plasma Antioxidant and MDA in Diabetic Patients.

Parameters	Normal Subjects	Recently Diagnosed	5 years	10 years	15 years	20 years
MDA (nmol/ml)	3.33±1.20	4.99±1.55*	6.14±1.29*	6.78±1.50	7.30±1.72*	7.56±1.47*
Vitamin C (µmol/L)	48.8±4.22	30.2±3.28*	31.0±3.62*	30.3±4.0*	27.7±2.10*	25.9±3.35*
Vitamin E (µmol/L)	19.4±1.12	16.9±0.96*	16.5±0.73*	15.9±0.76*	15.4±0.81*	15.5±0.96*
Thiol group (µmol/L)	533.6±31.4	415.5±23.4*	419.6±23.5*	427.0±17.3*	410.1±33.5*	382.4±25.7*
TRAPc (µmol/L)	956.5±53.9	793.9±47.0*	802.0±57.8*	804.4±44.1*	756.6±36.1*	707.8±53.5*
RBC GSH (mmol/gm Hb)	12.30±0.50	11.31±0.48*	10.89±0.37*	10.75±0.32*	10.27±0.45*	10.25±0.33*

*All values are mean ± SD. Total number of patients was 20 in each group. p value <0.05 is considered as significant. (*p<0.001, **p<0.01)*

Table 3 : Lipid Profile in Diabetic Patients and their Age / Sex Matched Healthy Controls.

Parameters (mg/dl)	Normal Subjects	Recently Diagnosed	5 years	10 years	15 years	20 years
Cholesterol	173.3±16.1	200.3±25.8*	196.8±22.7*	206.7±23.6*	199.6±27.0*	202.4±32.7*
Triglyceride	106.2±19.2	162.3±38.8*	149.4±46.8*	182.3±55.9*	164.1±44.8*	155.7±29.8*
HDLC	54.2±5.0	47.2±8.69**	46.8±7.27*	44.2±9.02*	43.5±9.44*	41.1±7.10*
LDLC	97.8±16.0	120.9±27**	120.1±22.2**	126.0±25.4*	123.3±27.1*	133.1±31.8*
VLDL	21.3±3.85	33.2±8.43*	29.9±8.98*	36.6±10.95*	32.8±8.97*	30.9±6.17*

*All values are mean ± SD. Total number of patients were 20 in each group. p value <0.05 was considered as significant. (*p<0.001, **p<0.01)*

From regression analysis, it was observed that though modest correlation exists between serum magnesium and glucose (r=-0.388, p<0.050) (Fig. 1), a highly significant positive correlation was observed between serum magnesium and TRAPc (r=0.66, p<0.005) (Fig.2) in the control subjects and all the diabetics taken together. This relationship was also correlation was not significant (Table 4).

In this study, we also found a strong correlation between serum and RBC magnesium (r=0.80, p<0.001) (Table 4). The correlation between RBC magnesium and other parameters was comparable with that of serum magnesium.

Fig. 1: Correlation of Serum Magnesium with Blood Glucose in Diabetic Patients and Normal Subjects

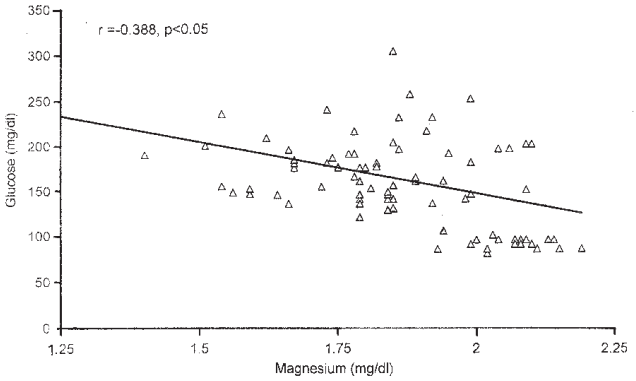


Fig. 2: Correlation of Serum Magnesium with TRAPc in Diabetic Patients and Normal Subjects

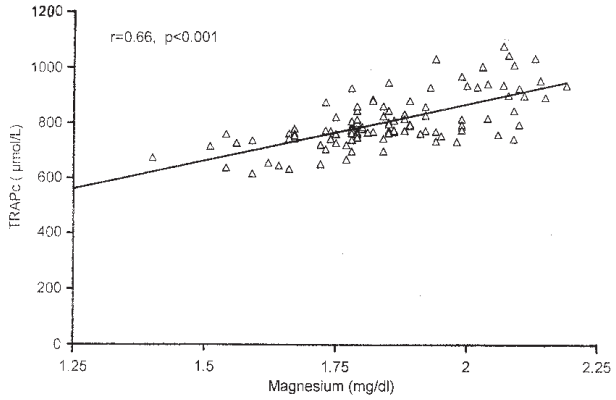
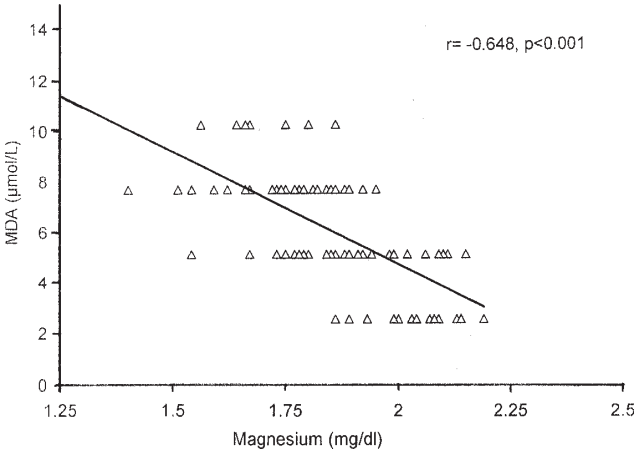


Table 4: Correlation Analysis of Serum Magnesium with other Parameters in Diabetic Patients and Normal Subjects taken together.

Parameters	Correlation Coefficient
RBC Mg	0.796*
Vitamin C	0.68*
Vitamin E	0.64*
Total thiols	0.648*
Uric acid	0.47***
RBC GSH	0.692*
Triglycerides	-0.25#
Cholesterol	-0.291#

*In normal subjects; n=20, in diabetic patients, n=100. 'p' value<0.05 was considered to be significant. (*p<0.001, ***pp<0.02, # non-significant).*

Fig. 3: Correlation of Serum Magnesium with MDA in Diabetic Patients and Normal Subjects



DISCUSSION

In the present study, it was observed that both serum and RBC magnesium levels are significantly lowered in diabetic patients as compared to their age and sex matched control subjects. Although plasma magnesium comprises only about 1% of total body magnesium, and may not reflect true picture of body magnesium status, nevertheless, in normal subjects, plasma magnesium levels are maintained within narrow limits by sensitive homeostatic mechanisms and the low values found in diabetic patients in this study at least imply that magnesium metabolism is commonly disturbed in diabetes. Moreover, plasma magnesium has been well correlated with intracellular free magnesium as reported by various studies (16,25) and a strong correlation between serum and RBC magnesium levels was observed in this study. The results presented here also show a definite relationship between serum magnesium concentration and duration of diabetes. The strong correlation of magnesium with duration of disease suggests that elderly diabetic patients are more susceptible to develop magnesium deficiency.

The mechanism responsible for magnesium deficiency in patients with diabetes is not completely known. Osmotic diuresis clearly accounts for a portion of the magnesium loss. The renal glycosuria that accompanies the diabetic state is believed to impair renal tubular reabsorption of magnesium from the glomerular filtrate (26). Other factors, however, including diarrhea, vomiting, sodium intake and diuretics use may play a role in magnesium

deficiency in diabetes mellitus. Insulin has been reported to enhance the transport of magnesium into cells; therefore, impaired insulin action or insulin resistance may result in an intracellular magnesium deficit (27).

In the present study, the plasma levels of malondialdehyde have been found increased in the diabetic patients and there was a significant reduction in the levels of plasma antioxidants. It has been suggested that the reduction in the antioxidant potential and increased free radical formation contributes to the development of oxidative stress in diabetes. Baynes (28) has proposed that oxidative stress may be a common pathway linking diverse mechanisms for the pathogenesis of complications in diabetes including non-enzymatic glycosylation of proteins, glucose auto-oxidation and enhanced polyol pathway activity. Even though it is well known that free radicals are capable of inducing the diabetic complications, however, how oxidative stress in diabetes initiates complications remains hypothetical.

It has also been reported that the plasma total antioxidant capacity is not determined by mere sum of the relative concentration of antioxidants, but is determined also by their synergism. Recently, the assay of the total plasma radical-trapping antioxidant parameter (TRAP) has been proposed to represent a more reliable estimation of plasma antioxidant capacity than the measurement of each known antioxidants (17). Cerriello et al (29) have found a strong correlation between TRAPm (measured directly by a fluorescence-based method) and TRAPc and proposed that since TRAPm is long and difficult to perform, TRAPc might be proposed to serve the purpose.

The principal finding in this study was the strong inverse relationship between serum magnesium and TRAPc in most of the diabetic patients. This has not been described previously in diabetes. The mechanism of inverse relationship between serum magnesium and TRAP may be attributed to the fact that magnesium deficiency itself is also associated with increased free radical-dependent oxidative stress (13). Regression analysis considering magnesium as the independent variable and malondialdehyde as dependent variable, showed a strongly significant correlation between these variables suggesting that magnesium deficiency might participate in the increased oxidative stress and decreased antioxidant potential. In fact it has been suggested that magnesium deficiency reduces antioxidant status in the cells so that they are more susceptible to free radical injury (14). It can therefore be proposed that changes in magnesium homeostasis might mediate the relation of oxidative stress to diabetic complications. Findings from other studies have implicated the role for increased oxidative stress during the tissue injury induced by magnesium deficiency (30).

Biological sequelae of hypomagnesemia in diabetic patients are not completely defined. It is possible that magnesium may be an important determinant of insulin sensitivity in non-insulin dependent diabetes mellitus. Magnesium deficiency has been linked to two most common complications of diabetes, namely retinopathy and ischemic heart disease (9,11). Hypomagnesemia can be a consequence of hyperglycemia and a cause of insulin resistance. In humans, insulin resistance has been implicated to impair the ability of insulin to stimulate magnesium or glucose uptake in diabetic individuals (27) thus suggesting that low serum magnesium could be a marker of insulin resistance and hyperinsulinemia. Tongyai et al (31) have suggested that low erythrocyte magnesium content can alter membrane viscosity, and this may impair the interaction of insulin with its receptor on the membrane. Thus low levels of magnesium may induce insulin resistance, which in turn attenuates magnesium uptake by insulin responsive tissues Magnesium may also be implicated in the development of diabetic complications via effects on inositol transport as suggested by Grafton et al (9).

Observations of dyslipidemia are also consistent with the other studies in that diabetes mellitus is associated with changes in the lipid metabolism. Magnesium deficiency has also been reported to affect the lipid metabolism (32). However, significant correlation between magnesium levels and cholesterol or triglycerides was not observed in this study.

Recognizing the signs of diabetes-induced magnesium deficiency is important, because the deficiency can occur long before it is reflected by the serum values. It could be demonstrated that there is a relationship between hypomagnesemia and late diabetic complications. Several large observational studies have demonstrated strong cross-sectional associations between low magnesium levels and type 2 diabetes. ARIC study has demonstrated strong cross-sectional association between low serum

magnesium levels and type 2 diabetes (33). This evidence stresses the concept of evaluating magnesium levels in serum of diabetic patients particularly with poor glycemic control. The main implication of our results is that low serum magnesium levels confer increased risk for diabetic complications through increased oxidative stress and altered lipid profile.

In conclusion, the data presented here suggests that diabetic patients are at increased risk of developing magnesium deficiency, which may play a role in insulin resistance and development of diabetic complications through increased oxidative stress. However, prospective studies are needed to demonstrate convincingly whether supplementation with magnesium will decrease the incidence of diabetes and its complications.

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