Assessment of magnesium status in newly diagnosed diabetic children: measurement of erythrocyte magnesium level and magnesium tolerance testing

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The aim of this study was to investigate the relationship between serum, erythrocyte and urine magnesium levels and retained magnesium percentage in newly diagnosed diabetic children. In a cross-sectional study, 34 children with insulin dependent diabetes mellitus (IDDM) and 21 healthy age- and sex-matched control subjects were screened for their serum, erythrocyte, and urine magnesium levels. Magnesium tolerance test was performed on diabetic and control subjects. Serum and erythrocyte magnesium levels in diabetic children were significantly lower than in healthy controls (plasma magnesium, p<0.05; erythrocyte magnesium, p<0.001); however, serum magnesium level was in normal range in diabetics and controls. Erythrocyte magnesium levels in diabetic children showed an inverse correlation with percentage of retained magnesium load (r=-0.44, p<0.01). Urine magnesium excretion in diabetic children (7.12±2.18 mmol/g creatinine/24-hr) was significantly higher than in healthy controls (4.0±1.35 mmol/g creatinine/24-hr) (p<0.001). There was a negative correlation between erythrocyte magnesium (2.07±0.62 mmol/L) and urine magnesium (7.12±2.18 mmol/g creatinine/24-hr) (r=-0.68 p<0.01) in diabetic children. Magnesium tolerance test showed that percentage of retained magnesium in diabetic children (66±26%) was significantly higher than in controls (16±7%) (p<0.001). This study is the first study to simultaneously investigate serum, erythrocyte and urine magnesium levels and magnesium tolerance test in newly diagnosed diabetic children. In conclusion, erythrocyte magnesium levels decrease earlier than serum magnesium in diabetic children. The follow-up parameters in diabetics may include the policy of monitoring magnesium status. Erythrocyte magnesium measurement is preferred to serum magnesium. Magnesium tolerance test is a reliable and sensitive method, which may be used as an alternative to erythrocyte magnesium measurement or in combination with it in hospitalized diabetic children.

Key words: erythrocyte magnesium levels, type 1 diabetes, magnesium tolerance test.

Insulin dependent diabetes mellitus (IDDM) in children is one of the most frequent chronic diseases causing hypomagnesemia. In several studies reduced magnesium concentrations have been observed in diabetic adults and children, despite a good nutritional status. Experimental studies have shown that hypomagnesemia inhibits prostacyclin receptor function, producing an imbalance between prostacyclin and thromboxane effects. Hypomagnesemia can increase platelet reactivity, increase vascular and adrenal responses to angiotensin II, enhance thromboxane A₂ (TXA₂) release, and lead to organ damage from free radicals. To date, the precise mechanism for the development of diabetic macrovascular changes is not clearly explained. Since the risk of ischemic heart disease is increased in diabetics, magnesium depletion may be one of the contributing factors to the development of ischemic heart disease in diabetics. Administration of magnesium has...
been shown to be protective against ischemic heart disease in animals with experimental magnesium deficiency. Adult diabetics with severe retinopathy have lower serum magnesium levels than diabetics without any obvious microvascular disorders.

Although several studies have shown that children with IDDM have significant bone loss, the pathogenic mechanism is not clearly understood. Serum 25-hydroxyvitamin D concentration is usually within normal range, and levels of the most active vitamin D metabolite, calcitriol, normal. Hypomagnesemia, demonstrated in most diabetic children, may affect both parathyroid function and 1-α-hydroxylase activity. Saggese et al. found lower serum values for total and ionized calcium, magnesium, intact parathyroid hormone (PTH), calcitriol, and osteocalcin in diabetic children. They concluded that magnesium supplement in diabetic children improved all these parameters. In addition, because magnesium is mainly an intracellular ion and involved many of the enzyme systems regulating intracellular glucose metabolism, decreasing the total body magnesium stores also produces insulin resistance.

To the best of our knowledge, there is currently no routine practice to monitor magnesium levels in diabetic children. In many studies, serum magnesium was measured to assess magnesium status in diabetic children. It is well known that serum magnesium level in diabetic patients is not predictive of intracellular content. The aim of this study was to assess magnesium status in serum, erythrocyte, and urine in diabetic children. To assess the deficit in total body magnesium store, magnesium tolerance test was carried out and magnesium supplement commenced based on the result of the test.

Material and Methods

Subjects

A total of 37 children with IDDM were included in the study. Patients and control characteristics are given in Table 1. Twelve patients were newly diagnosed in our hospital between January 1998 and March 2003, and 17 patients were referred from state and social security hospitals in Düzce and neighboring cities. Three patients dropped out following the Düzce earthquake in September 1999. All children with IDDM were treated with insulin infusion for the first 24-48 hours, and subsequently with two doses of short- and intermediate-acting insulin (Mixtard, Novo Nordisk, Copenhagen, Denmark; Humulin M, Eli Lily, Indianapolis, USA). They were managed according to the general principles currently in practice in Turkey, including a diet intended to provide 15-20% of the energy as proteins, 30-35% as fats and 5-50% as carbohydrates. Metabolic control was assessed by self-monitoring glucose records at home, and by fasting plasma glucose and hemoglobin A1c (% (HbA1c, %) levels every three months in hospital. All children had good or reasonable metabolic control. Twenty-one-age, and sex-matched healthy children were considered as control subjects. Informed consent was obtained from parents or guardians of diabetic and control subjects. The ethical committee of Düzce Medical Faculty approved the study protocol for investigation in humans.

Table 1. Clinical Data in Children with Insulin Dependent Diabetes Mellitus (IDDM) and in Control Subjects

<table>
<thead>
<tr>
<th>IDDM</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>34</td>
</tr>
<tr>
<td>Gender ratio (M/F)</td>
<td>19:15</td>
</tr>
<tr>
<td>Age (yr)*</td>
<td>10.0 (6-17)</td>
</tr>
<tr>
<td>Duration of IDDM (mo)*</td>
<td>5.4±0.3</td>
</tr>
<tr>
<td>Insulin (IU/kg/day)*</td>
<td>0.9 (0.6-1.3)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.2±0.6¶</td>
</tr>
</tbody>
</table>

* Values expressed as median (range).
¶p<0.001 versus control subjects (Student’s t test).

Materials

The study was commenced after the recovery period of diabetic ketosis or ketoacidosis, usually within the first six months of diagnosis. Blood samples were obtained after an overnight fasting period, between 08.00-09.00 a.m. in the fasting condition and before the morning insulin injection in acid-rinsed, metal-free glass test tubes for measurement of plasma glucose, serum magnesium and HbA1c levels. Two-ml blood sample was taken in metal-free heparinized plastic tubes and centrifuged for measurement of magnesium levels in erythrocytes. Twenty-four hour urine specimens were collected in metal-free containers and acidified to pH 1.0. After the collection of the 24-hr urine specimen, magnesium sulfate (MgSO₄.7H₂O), in a 50% solution (5 ml
containing 20.3 mEq of elemental magnesium), was administered by an intravenous infusion in a dose of 0.2 mEq of elemental magnesium per kg body weight in 50 ml of 5% dextrose over four hours. The second 24-hr urine specimens (starting with infusion) were collected for urinary magnesium, creatinine, and albumin measurement. Percentage of the retained magnesium was calculated by the following formula:

\[ \text{Magnesium retained} \% = 1 - \left( \frac{(A - [B \times C])}{D} \right) \times 100 \]

A, postinfusion 24-hour urine magnesium
B, preinfusion 24-hour urine magnesium/creatinine
C, postinfusion urine creatinine
D, total elemental magnesium infused

Interpretation of test results: greater than 50% retention at 24 hours indicates definite deficiency; between 25% and 50% retention at 24 hours indicates probable deficiency; lower than 25% retention indicates normal body magnesium stores.

**Laboratory Analysis**

Magnesium content in erythrocytes was evaluated by washing them twice in 0.9% saline solution at 4°C; after the last centrifugation, the packed cells were decanted into a 10-ml metal-free thin-walled container. The pallet was resuspended in double-distilled water for breaking of erythrocytes. Released magnesium was measured by atomic absorption spectrophotometer (Perkin-Elmer 403, Norwalk, USA). Plasma and urine magnesium was also measured by atomic absorption spectrophotometer. The HbA1c was measured by the method of turbidimetric inhibition immunoassay using Tina/quant hemoglobin A1c II kit (Roche Diagnostics GmbH, D-68298 Mannheim, Germany). According to the reference values of HbA1C for the Tina-quant HbA1C assay, between 4.8% and 6.0% is normal, between 6.1% and 7% is an indication of ideal control in diabetic patients, between 7.0% and 8.0% is reasonable control, and >8.0% is poor control. Fasting plasma glucose and serum and urine creatinine were measured by an autoanalyzer (DPC, Los Angeles, USA). Urine albumin excretion was monitored by urine dipstick (Redia test®, Boehringer Mannheim, GmbH, Mannheim, Germany).

**Statistical Analysis**

Statistical analysis was performed using the SPSS/PC package, version 10.0 (SPSS, Chicago, USA). Results were expressed as means±SD. Comparison of means between diabetic and control subjects was analyzed by Student’s t-test. Pearson’s test was used to calculate correlation among the measurements.

**Results**

None of the children exhibited clinical signs of vascular disease or albuminuria. Their total daily median insulin dosage was 0.9 (0.6-1.3) IU per kg body weight. Serum, erythrocyte, and urine magnesium levels and retained magnesium percentage in magnesium tolerance test are highlighted in Table II. However, serum magnesium levels were in normal range in diabetic children (0.79±0.12 mmol/L) and in control subjects (1.19±0.23 mmol/L), whereas there was a greatly reduced mean erythrocyte magnesium level in diabetic children (2.07±0.62 mmol/L) as compared with healthy controls (5.14±0.72 mmol/L) (p<0.001) (Table II). There was a significant positive correlation between serum magnesium and erythrocyte magnesium (r=0.375, p<0.05). Urine magnesium levels in diabetic children (7.12±2.18 mmol/g creatinine/24-hr) was

| Table II. Serum Erythrocyte, and Urine Magnesium Levels and Retained Magnesium Percentage in Magnesium Tolerance Test |
|-------------------------------------------------|-------------------------------------------------|------------------|
| Serum magnesium levels (mmol/L)                | Control group (n=21)                            | P*               |
| (n=34)                                          |                                                  |                  |
| 0.79±0.12                                       | 1.19±0.23                                       | <0.05            |
| Erythrocyte magnesium levels (mmol/L)           |                                                  |                  |
| 2.07±0.62                                       | 5.14±0.72                                       | <0.0001          |
| Urine magnesium levels (mmol/g creatinine/24 hr)|                                                  |                  |
| 7.12±2.18                                       | 4.0±1.35                                        | <0.05            |
| Retained magnesium (%)                          |                                                  |                  |
| 66±26                                           | 16±7                                            | <0.0001          |

* difference is significant at the 0.05 levels.
significantly higher in control subjects (4.0±1.35 mmol/g creatinine/24-hr) (p<0.001). Magnesium tolerance test showed that 15 of the diabetic children (44%) retained greater than 50% of loaded magnesium, and 16 (47%) children retained between 25% and 50% of loaded magnesium. Retained magnesium percentage was significantly higher in diabetic children (66±26%) than in the control group (16±7%) (p<0.001) (Fig. 1). There was a significant inverse correlation between 1) erythrocyte magnesium levels and urine magnesium levels (r=-0.685, p<0.05) 2) erythrocyte magnesium levels and retained magnesium levels percentage (r=-0.446, p<0.01), and 3) erythrocyte magnesium levels and age (r=-0.348, p=0.04). There was no correlation between urine magnesium concentration and HbA1c % or fasting plasma glucose level, and sex differences were not found to be correlated for any variable in this study.

Discussion

The etiology of hypomagnesemia in diabetes cannot be clearly explained. Magnesium deficiency in humans is unlikely to occur from a simple lack of foods containing this mineral, except in advanced forms of malnutrition. According to the consensus of a panel on magnesium metabolism in diabetes mellitus, diabetic patients have additional risk factors for hypomagnesemia and magnesium status, which should be evaluated regularly. However, there is no consensus on the evaluation methods to determine magnesium status in diabetic children. Previous studies used serum magnesium, intracellular and extracellular magnesium, urinary magnesium excretion, and magnesium tolerance test to assess magnesium status in diabetic children. The most important finding of this study was erythrocyte magnesium levels at significantly lower levels in newly diagnosed diabetic children than in control subjects. A high percentage of retained magnesium confirmed that newly diagnosed children were magnesium-deficient, although serum magnesium levels were in normal ranges. One explanation is that the pre-treatment period of diabetes, usually associated with osmotic diuresis and excessive loss of magnesium, causes the deficit of cellular magnesium stores. But the magnesium levels in diabetic patients have a tendency to continuously decrease, even if the patients are in good metabolic control or have regularly balanced diets. To the best of our knowledge, the present study is the first to consider combination of intracellular and extracellular magnesium levels, urine magnesium levels, and magnesium tolerance test. Serum magnesium levels are not affected in the early period of diabetes, usually associated with osmotic diuresis and excessive loss of magnesium, which is the only reliable index of total body magnesium. Our findings showed that the erythrocyte magnesium level is affected as early as six months after the diagnosis of diabetes, and is therefore a more sensitive marker to assess magnesium status in diabetic patients. Assessment of magnesium status and the need for magnesium supplementation in diabetics may be based on erythrocyte magnesium levels rather than on serum magnesium.

Magnesium tolerance test has been utilized for the assessment of tissue magnesium stores. In our study, magnesium tolerance tests revealed a definite magnesium deficiency in 15 diabetic children and probable deficiency in the other 16 diabetic children. Only three patients’ total magnesium body stores were considered normal. These findings suggest that magnesium tolerance testing is a reliable and sensitive method to monitor magnesium status in
diabetic children. However, according to the testing procedure, the requirements for two different 24-hour urine samples and a short period of hospitalization for parenteral magnesium loading are limiting factors in routine practice. However, this may be conducted when the diabetic children are hospitalized with the other indications, or to consider whether magnesium supplementation is required.

The mechanisms of long-term complications of diabetes are not clearly explained, and hypomagnesemia may be contributing factor to these complications, particularly ischemic heart disease$^{10-14}$, retinopathy$^{1,15}$ and bone loss$^{22-24}$. Therefore, magnesium level should be monitored in routine practice in diabetic children. At a minimum, erythrocyte magnesium should be measured every three to six months. If erythrocyte magnesium is detected at low levels, magnesium supplementation is required.

In conclusion, magnesium is one of the important elements for diabetics and is decreased, usually by an unexplained mechanism. It is claimed that chronic hypomagnesemia has an effect on insulin resistance, and macrovascular and microvascular complications in diabetics. In addition, hypomagnesemia is the most important etiological factor that causes bone loss in diabetics. The follow-up parameters in routine practice in diabetics may include the policy of monitoring magnesium status. Erythrocyte magnesium measurement should be checked every three to six months. If erythrocyte magnesium is detected at low levels, magnesium supplementation might be given$^{24,32}$. In hospitalized patients, magnesium tolerance test can be conducted to interpret the total magnesium stores.

REFERENCES


