Serum Trace Elements in Type 2 Diabetes Mellitus

Hande Atelay1, Banu Boyuk1, Savas Guzel1, Murat Altay2, Ali Riza Kiziler3, Birsen Aydemir5

1Gebze Fatih Government Hospital, Internal Medicine Department, Kocaeli, Turkey - 2Department of Internal Medicine, Gaziosmanpasa Taksim Education and Research Hospital, Turkey - 3Department of Biochemistry, Namik Kemal University, Turkey - 4Department of Biophysics, Namik Kemal University, Turkey - 5Department of Biophysics, Medical Faculty, Sakarya University

ABSTRACT

Studies suggested that imbalances of several trace elements may play an important role in normal glucose and insulin metabolism. The aim of the present study was to evaluate changes in serum levels of copper, zinc, iron, and magnesium in patients with type 2 diabetes mellitus (T2DM) and their effect on glycemic control. Sixty female patients with T2DM and seventeen healthy subjects were enrolled in this study. Fasting plasma samples were obtained from the patient and control groups. Trace elements were studied using an atomic absorption spectrometer. Correlation analyses of trace elements with metabolic parameters were analyzed using Spearman’s Rho correlation coefficient. T2DM patients had a significantly high fasting plasma glucose, glycated hemoglobin (HbA1C), and microalbuminuria levels (p<0.05). Serum magnesium levels were significantly lower in patients with T2DM compared with the healthy controls (2.0±0.2 ng/mL vs. 2.3±0.2 ng/mL, p<0.05). Serum copper levels showed a negative correlation with diabetes duration (r= -0.338, p=0.011), and iron levels were negatively correlated with body mass index and C-reactive protein (r= -0.407, p=0.009; r= -0.390, p=0.017). Serum magnesium levels indicated a correlation with HbA1C and creatinine clearance (r= -0.371, p=0.049; r= -0.462, p=0.023), but no significant correlation was found with any of the other variables and zinc levels.

The present study found low levels of magnesium, iron, copper, and zinc in women with T2DM, which supports a close relationship of the above trace elements with glucose metabolism. Low magnesium levels has been linked to poor glycemic control in T2DM; therefore, magnesium deficiency should be prevented in patients with diabetes.

Keywords: Diabetes, trace elements, HbA1C.

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Introduction

Type 2 diabetes mellitus (T2DM) is a chronic disorder of glucose homeostasis associated with insulin deficiency or/and resistance to the action of insulin. Chronic hyperglycemia leads to microvascular complications such as nephropathy, retinopathy, and neuropathy41. Trace elements are required in small quantities for specific functions in the body. Clinical research seems to show a link between trace elements and glucose homeostasis. Studies suggested that imbalances of several trace elements may play important roles in glucose and insulin metabolism, and chronic hyperglycemia may cause significant alterations in the status of some trace elements2,3. In addition, disturbances in trace element status and increased oxidative stress in diabetes mellitus may contribute to the development of diabetic complications42. Copper (Cu) deficiency was an indicator of impaired glucose tolerance in an earlier study43. Zinc (Zn) homeostasis affects the synthesis, storage, and secretion of
insulin, and glycemic control as a result\(^6\). High iron (Fe) levels were linked to insulin resistance and were reported to have contributed to an increase in T2DM incidence\(^7\). Hypomagnesemia has a negative impact on glucose homeostasis and insulin sensitivity in humans\(^8\).

In the light of previous studies, we aimed to evaluate changes in serum levels of Cu, Zn, Fe, and magnesium (Mg) in patients with T2DM and their effect on glycemic control and microalbuminuria.

**Materials and methods**

**Study population**

Sixty women with T2DM and twenty healthy subjects were enrolled in this study between January and June 2014. The diagnosis of diabetes was based on a previous diagnosis of T2DM or a random plasma glucose level of 200 mg/dL or higher, together with classic features of DM, such as polyuria, polydipsia, polyphagia, and weight loss, or a fasting blood glucose level of >126 mg/dL or glycated hemoglobin (HbA1C) levels of 6.5% or higher. The exclusion criteria were as follows: treatment with vitamin and mineral supplements, history of any recent acute illness or clinical evidence suggestive of kidney, liver, or endocrine diseases, digestive disorders, non-normal dietary habits, and any weight change in the previous 6 months. The study protocol was approved by the ethics committee of GOP Taksim Research and Education Hospital, Istanbul.

**Measurements**

Hypertension was defined as antihypertensive drug use or systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg. Body mass index (BMI) was obtained using the formula weight (kg)/height (m\(^2\)). Obesity was defined as a BMI ≥30 kg/m\(^2\). Fasting plasma samples were obtained from the patient and the control group. Serum cholesterol, triglyceride, and high-density lipoprotein cholesterol (HDL-C), albumin, parathormone, calcium, and phosphorus were measured using enzymatic colorimetric methods with commercially available kits (COBAS 311, Roche Diagnostics GmbH, Mannheim, Germany) and low-density lipoprotein cholesterol C (LDL-C) was calculated according to the Friedewald formula. Serum glucose measures were determined enzymatically using the hexokinase method (Roche Diagnostics GmbH, Mannheim, Germany).

Blood HbA1c was determined using a COBAS 311 analyzer using the particle-enhanced immunoturbidimetric method (Roche Diagnostics, Mannheim, Germany). The final results are expressed as percent HbA1c of the total hemoglobin (Hb) according to the protocol of the Diabetes Control and Complications Trial/National Glycohemoglobin Standardization Program (DCCT/NGSP). The particle-enhanced immunoturbidimetric method was used with a Behring Nephelometer BN-100 (Behring Diagnostic, Frankfurt, Germany) to measure C-reactive protein (CRP). The glomerular filtration rate (GFR) was estimated using 24-hr urine creatinine clearance, and renal failure was defined as GFR <60 mL min\(^{-1}\) per 1.73 m\(^2\). Microalbuminuria was investigated in 24-hr urine samples. Five-milliliter venous blood samples were drawn from each patient in a metal-free sterile tube after 8-hr fasting conditions. The blood samples were then kept at room temperature for about 30 min to clot and centrifuged at 3000 rpm for 15 min to extract the serum. The serum was taken in Eppendorf tubes and stored at -80°C until the study day. Determination of trace elements was conducted using flame atomic absorption spectrometry (Shimadzu Japan AA-6800). The samples were diluted with deionized water by a dilution factor of 10. Different concentrations (0.5, 1.0, 2.0, 5.0, and 10.0 mg/L) of trace elements were used for the calibration of standard graphs. Absorbances were taken at 213.9, 224.8, 279.8, and 248.3 nm for Zn, Cu, Mg, and Fe, respectively, in the atomic absorption spectrometer. To verify the assay accuracy and to maintain quality, a standard solution was run after every ten samples.

**Statistical analyses**

Compliance with normal distribution for continuous variables was analyzed using the Shapiro-Wilk test. Descriptive statistics are used to describe continuous variables (mean, standard deviation, minimum, median, maximum) percentage (%) and N are used to identify dashed variables. Student’s t-test was used to compare independent and normally distributed continuous variables between the two groups. Student’s t-test was used to compare continuous variables showing independent and normal distribution. The Mann-Whitney U test was used to compare continuous variables showing independent and normal distribution. The relationship between discrete variables was tested using the Chi-square test.
The correlation between continuous variables showing abnormal distribution was examined using Spearman’s Rho correlation coefficient. Statistical significance was set at P=0.05. Analyses were performed using MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org, 2013).

**Results**

The patient group included 60 women and the control group comprised 20 women. The clinical and biochemical characteristics of the patients and controls are shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1: Clinical and biochemical characteristics of patients and controls.</th>
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<tr>
<td><strong>Patient group (n=60)</strong></td>
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<tr>
<td><strong>Age (years)</strong></td>
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<tr>
<td><strong>Diabetes duration (years)</strong></td>
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<td><strong>BMI (kg/m2)</strong></td>
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<td><strong>Systolic pressure (mmHg)</strong></td>
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<td><strong>Diastolic pressure (mmHg)</strong></td>
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<td><strong>Fasting blood glucose (mg/dL)</strong></td>
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<td><strong>HbA1c (%)</strong></td>
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<td><strong>Triglyceride (mg/dL)</strong></td>
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<td><strong>Total cholesterol (mg/dL)</strong></td>
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<td><strong>LDL (mg/dL)</strong></td>
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<td><strong>HDL (mg/dL)</strong></td>
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<td><strong>CRP (mg/L)</strong></td>
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<td><strong>Creatinine clearance (ml/min)</strong></td>
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<td><strong>Microalbuminuria</strong></td>
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<tr>
<td><strong>Cu</strong></td>
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<td><strong>Zn</strong></td>
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<td><strong>Fe</strong></td>
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<td><strong>Mg</strong></td>
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The serum Cu, Zn, Fe, and Mg levels were significantly lower in patients with T2DM compared with the healthy controls (p<0.05). The patients with T2DM had significantly lower creatinine clearance than the control subjects (p<0.05). There were no significant differences in other variables between the patients with T2DM and controls.

Serum Cu levels showed a negative correlation with diabetes duration (r= -0.338, p=0.011), and Fe levels were negatively correlated with BMI and CRP (r= -0.407, p=0.009; r= -0.390, p=0.017), and positively correlated with HDL (r=0.632, p=0.007). Serum Mg levels indicated a negative correlation with HbA1C and fasting blood glucose.

**The mean duration of diabetes was 9.7±8.7 years.** The patients with T2DM had significantly higher fasting plasma glucose, HbA1C, and microalbuminuria levels than the control subjects (p<0.05).
Mg values with other parameters.

**Table 3**

<table>
<thead>
<tr>
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<th>Cu</th>
<th>Fe</th>
<th>Mg</th>
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</thead>
<tbody>
<tr>
<td>R</td>
<td>-0.38</td>
<td>0.041*</td>
<td>0.019</td>
</tr>
<tr>
<td>p</td>
<td>0.011</td>
<td>0.908</td>
<td>0.091</td>
</tr>
<tr>
<td>r</td>
<td>-0.20</td>
<td>0.009*</td>
<td>0.007*</td>
</tr>
<tr>
<td>p</td>
<td>0.026</td>
<td>0.183</td>
<td>0.049</td>
</tr>
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**Diabetes duration**

Linear regression analysis was used to assess Cu, Fe, and Mg values with the other parameters for all patients with T2DM. We found that serum copper levels were negatively associated ($\beta = -0.338$) with the duration of T2DM and serum Fe levels were positively associated ($\beta = 0.335$) with triglycerides. Serum Mg correlated negatively ($\beta = -0.451$) with levels of HbA1C in all patients with T2DM (Table 3).

Table 2: Association of serum Cu, Fe and Mg levels with metabolic parameters.

Pearson correlation *p<0.05 Cu: Copper, Fe:Iron, Mg:Magnesium

Cu: Copper, Zn: Zinc, Fe:Iron, Mg:Magnesium

Discussion

Many studies reported that trace elements were linked to diabetes and its complications. The present study has shown that serum Mg, Cu, Zn, and Fe levels were significantly lower in women with T2DM when compared with healthy controls. Lower blood concentrations of Mg have been shown in patients with T2DM than in nondiabetic subjects in the literature. Our results are in accordance with the literature in that the patients with T2DM had low serum Mg levels and these correlated with HbA1C levels. The low levels of Mg in patients with T2DM can be explained through insufficient dietary intake, opposite change of erythrocyte and plasma Mg due to hyperglycemia, use of loop and thiazide diuretics or decreased tubular reabsorption due to insulin resistance. Hadjistavri LS et al. suggested that Mg supplementation improved insulin sensitivity and beta cell function. Nadler JL et al. demonstrated that low magnesium diets impaired insulin sensitivity after just 3 weeks. Overall, low magnesium levels seem to be associated with a lower risk of T2DM.

Copper is another trace element that is primarily needed for the activity of superoxide dismutase (SOD) and cytochrome oxidase. However, the data about Cu and its effect on glucose metabolism or the way its deficiency influences diabetes are limited in the literature. A few studies reported that male patients with diabetes had increased serum Cu levels but female patients had normal levels. Pidduck HG et al. reported that serum Cu levels ranged from normal in diabetics.

Our results demonstrated that women with diabetes had significantly lower plasma Cu levels than healthy women, and serum Cu levels were negatively associated with the duration of T2DM. Low plasma Cu levels may induce diabetes by resulting in oxidative stress. Lower levels of blood Zn in patients with T2DM were documented in the literature. According to Kazi et al., lower levels of Zn were linked to increased urinary excretion of Zn, especially in patients with diabetic complications such as nephropathy. Similarly, the findings of our study revealed significantly lower levels of serum Zn in women with T2DM compared with controls. However, no correlation was found between Zn and metabolic parameters in patients with T2DM. Zinc is known as an anti-inflammatory and antioxidant element. A recent study demonstrated downregulation of inflammatory markers with Zn supplementation in the elderly. However, further investigations are necessary to understand the effect of zinc supplementation on glycemic control of patients with diabetes.

Iron was recently suggested to affect glucose metabolism, in addition to its main role in hemoglobin and myoglobin synthesis. It is hypothesized that iron and glucose homeostasis link at multiple levels. Furthermore, iron supplementation should be a potential target therapy for patients at risk for T2DM. On the other hand, serum iron and ferritin levels were correlated with insulin and glucose concentration, and related with poor glycemic con-
trol in the patients with T2DM in some studies\(^{30-33}\). In our study, iron levels were negatively correlated with BMI and CRP, and positively associated with triglycerides. However, in contrast to previous studies, no association was demonstrated with glycemic control.

Our research demonstrated a significant decrease in serum Mg levels only in patients with microalbuminuria, but not for Zn, Cu or Fe. A decline in kidney function as observed by creatinine clearance levels in subjects with T2DM was associated with a decrease in serum Mg levels \(^{32}\). Decreased serum Mg patients with T2DM with poor kidney function was also reported in other trials\(^{33,34}\). A study documented a significant increase in urinary magnesium excretion in subjects with T2DM\(^{40}\). Low Zn and Cu levels in patients with diabetic nephropathy have also been documented in the literature\(^{46}\). These inconsistent findings may be the result of different amounts of elements in diet, individual differences in absorption, and utilization.

This study has some limitations. First, the sample size is small thus the generalizability of our findings is limited. Second, because this is a cross sectional study, the dietary amount of trace elements is not known.

In conclusion, our results support previously reported interactions of T2DM with Mg, Cu, and Zn; however, the results are conflicting regarding the link between iron and diabetes. The role of trace elements in the pathogenesis of diabetes and the effect of trace element supplementation on glycemic control and microalbuminuria in T2DM needs to be investigated in further large-sample-sized population-based studies.

References


Corresponding author
HANDE ATALAY, M.D.
Gebze Fatih Government Hospital, Internal Medicine Department, Kocaeli (Turkey)