Assessment of serum calcium, magnesium and zinc levels in patients with type 2 diabetes mellitus in the Ukrainian population


Abstract. Background. The research deals with the provision of calcium, magnesium and zinc in patients with type 2 diabetes mellitus (T2DM). The purpose was to investigate the serum content of calcium, magnesium and zinc in patients with type 2 DM. Materials and methods. The open-label controlled study included 27 patients with T2DM. The control group consisted of 12 people without endocrine and somatic diseases. 70% of patients had newly diagnosed DM; in others, the disease duration was up to 5 years. Most of the examined were of mature age, on average 61.83 ± 5.52 years for patients and 55.25 ± 5.52 years for controls. In the group with T2DM, women accounted for 62.96%, among controls — 63.64%. Anthropometric measurements and glucose indicators were determined according to a standard procedure. Concentrations of calcium, magnesium, zinc in serum were evaluated by atomic optical emission spectrometry with inductively coupled plasma (PerkinElmer Optima 2100 DV ICP-OES, USA) according to the original procedure approved by the Kundiiev Institute of Occupational Health of the NAMS of Ukraine. Results. The level of fasting blood sugar ranged from 7.1 to 17.2 mmol/l and indicated the presence of T2DM, glycated hemoglobin was from 7.1 to 11.2%. According to anthropometric data, the group of patients almost completely corresponded to the control group. Although a slight increase in body weight and waist circumference was registered already at the stage of initial diabetes, the level of triglycerides increased significantly, and the level of high-density lipoprotein decreased. A significant reduction in the levels of calcium and magnesium in the blood serum of patients with T2DM was found. The zinc content was at the level of the lower edge of the reference value in 44% of patients, in the control group — only in 16.6% of cases. There were no significant differences in this indicator in the observation groups. A high correlation was found between the studied parameters in the blood, as well as an inverse correlation between the fasting glucose level and the content of calcium and magnesium. Conclusions. A significant decrease in the level of calcium and magnesium in the blood of Ukrainian patients with type 2 diabetes mellitus was found. There is a high correlation between serum calcium, magnesium and zinc levels in patients with T2DM. Significant risks of diabetes were revealed when the serum concentration of calcium and magnesium decreased.

Keywords: type 2 diabetes mellitus; fasting blood glucose; calcium; magnesium; zinc; correlation analysis

Introduction

Type 2 diabetes mellitus (DM) is a highly prevalent, costly, chronic disease that poses a serious threat to both domestic and global health [1]. The International Diabetes Federation reports that 10% of adults (aged 20—79 years) have type 2 DM worldwide [2]. Type 2 DM is currently one of the leading causes of death in the USA with a crude death rate of 26.7 per 100,000 people [3]. A common and potentially preventable disease, type 2 DM imposes substantial health and economic burdens in the USA, where the medical costs and lost work wages of people diagnosed with the disease total an estimated $327 billion annually [4].
The most prevalent complications of type 2 DM include microvascular events, including neuropathy, nephropathy, and retinopathy, or macrovascular events such as atherosclerosis, aneurysm, embolism, peripheral vascular diseases, cerebrovascular disease, or coronary artery disease [5]. Half of people diagnosed with type 2 DM present microvascular complications, and 27 % have macrovascular complications [6]. Chronic kidney disease is one the most common and devastating complications of DM, with 20–40 % of all persons diagnosed with type 2 DM developing kidney disease. Diabetic foot is another well-known complication of uncontrolled diabetes affecting 4–10 % of people diagnosed with type 2 DM. Other complications include diabetic retinopathy, cataracts, erectile dysfunction, and non-alcoholic fatty liver disease [7].

Trace elements refer to any chemical element indispensable for various vital activities of our body. These microelements are required in small amounts by the body, but their absence can lead to significant negative impacts on the health and survival of organisms. There are some indications that the pathophysiology and the occurrence of DM are related to alterations in serum trace elements [8]. Patients with DM are generally diagnosed with abnormal levels of serum trace elements, including magnesium, calcium, zinc, selenium, and others. In most cases, these microelements are pathologically lost or reduced in vivo [9].

The maintenance of optimal micronutrient levels is essential for glucose homeostasis. Remodeling the function of pancreatic islets and restoring the glucose homeostasis denote an ultimate cure for DM, but it remains a great challenge due to mutations in the glucose metabolism profile. Microelements supplementation, especially using their nanoparticle form, is showing unique therapeutic vision in DM [10].

Calcium (Ca$^{2+}$) is a highly versatile intracellular signal capable of regulating different cellular processes. The role of intracellular calcium as a mediator of insulin action was originally proposed [11]. Changes in concentration of free Ca$^{2+}$ in different compartments (cytosol, endoplasmic reticulum, mitochondria, Golgi, nucleolus, secretory vesicles, endo-/lysosomes) are crucial for the control of many cellular responses. The optimal concentration of cytosolic Ca$^{2+}$ appears to be within the range 140–350 nM. When Ca$^{2+}$ concentrations are too low or too high, the ability of pancreatic islets and insulin target cells to respond appropriately to physiological stimuli is significantly diminished leading to a break on glycemic homeostasis [12].

Magnesium (Mg) is one of the most abundant ions in the human body and is essential for many physiological processes. For example, Mg is a cofactor of hundreds of enzymes involved in cellular bioenergetics, protein metabolism, and cell signaling pathways. Thus, Mg deficiency in the development and persistence of type 2 DM. Although Mg deficiency has been widely studied in type 2 DM, the molecular clinical and molecular mechanisms are poorly reviewed [13].

DM has been linked to disruptions of zinc homeostasis. Zinc is an essential trace metal that regulates many enzymatic functions and cellular processes in the human body, including apoptosis, oxidative homeostasis, immune function, biological control of metabolism as well as signal transduction [14]. Zinc supplementation has been reported to exhibit beneficial effects in enhanced glycemic control in diabetic animals and humans. Furthermore, this metal can also improve the conditions associated with diabetic complications such as nephropathy and cardiomyopathy [15].

The study of these elements in patients with type 2 DM provides an opportunity to improve their treatment. This research purposed to investigate the serum content of calcium, magnesium and zinc in patients with type 2 DM.

**Materials and methods**

An open-label randomized controlled trial was conducted to evaluate the serum content of calcium, magnesium and zinc in 27 patients with type 2 DM aged 18–79 years. The control group included 12 persons without endocrine and somatic diseases. Written informed consent was taken from all patients to participate in this study. We included studies of adult humans with type 2 DM. We excluded studies of patients with type 1 DM, impaired glucose tolerance, cardiovascular complications.

The study protocol was conducted according to the principles of the Declaration of Helsinki and approved by the local Ethical Committee from the SI “V.P. Komisarenko Institute of Endocrinology and Metabolism of the NAMS of Ukraine”. Informed consent was obtained from all participants.

Data on age (year), duration of DM (months), treatment of DM, anthropometrics (body weight, height), and clinical and analytical data were recorded. Body weight and height were determined using certified electronic scales (KERN 440-4, Kern & Sohn, Germany) and a portable stadiometer. Weight was measured to the nearest 0.1 kg, height to the nearest 0.1 cm. Body mass index (BMI) was calculated as BMI (kg/m$^2$) = (weight in kg) / (height in meters). Waist circumference (WC) was measured using a flexible centimeter tape with a measurement accuracy of 0.5 cm at the level of the maximum waist size in a standing position.

Fasting blood samples were collected to measure glucose, insulin, calcium, magnesium, zinc serum, total cholesterol, triglyceride, and glycated hemoglobin (HbA1c). Serum calcium, magnesium and zinc levels were analyzed by atomic optical emission spectrometry by inductively coupled plasma (PerkinElmer Optima 2100 DV ICP-OES, USA) according to the original procedure approved by the Kundiev Institute of Occupational Health of the NAMS of Ukraine. Multi-element standard solution (Merck, Germany, catalog number 111355.0100) containing 23 elements was used to build a calibration curve for Ca, Mg and Zn in the ICP-OES analysis. Reference ranges for magnesium, calcium, zinc were considered respectively as 17–28, 90–112, 0.6–1.2 mg/L [16].

Statistical data processing was performed in accordance with the requirements of evidence-based medicine and biostatistics, using the approaches of modern non-infectious epidemiology [17]. The statistical package used the software package SPSS 16.0 and MedStat. Student and Mann–Whitney criteria were used for odd comparisons.

The experimental data were given as mean ± SE, median, and quartiles due to their non-normality of distribution of experimental variables. The correlations between experimen-
Table 1. Results of studies of parameters of diabetes and calcium, magnesium, zinc in patients with type 2 DM and in controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Type 2 DM (n = 27)</th>
<th>Control group (n = 12)</th>
<th>P (type 2 DM vs controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women, %</td>
<td>37.04</td>
<td>36.36</td>
<td>1</td>
</tr>
<tr>
<td>Age, years</td>
<td>61.83 ± 1.93</td>
<td>55.25 ± 5.52</td>
<td>0.16</td>
</tr>
<tr>
<td>FPG, mmol/l</td>
<td>10.16 ± 0.528</td>
<td>5.320 ± 0.136</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>7.7 (6.8–9.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>166.140 ± 1.785</td>
<td>166.100 ± 2.278</td>
<td>0.991</td>
</tr>
<tr>
<td>BW, kg</td>
<td>93.15 (83.25–107.78)</td>
<td>79.5 (75.5–97.25)</td>
<td>0.052</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>35.33 ± 1.33</td>
<td>31.07 ± 2.24</td>
<td>0.105</td>
</tr>
<tr>
<td>WC, cm</td>
<td>113.38 ± 2.89</td>
<td>100.2 ± 5.4</td>
<td>0.028</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>142.89 ± 3.75</td>
<td>129.3 ± 7.8</td>
<td>0.087</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>81.0 (79.0–90.0)</td>
<td>80.0 (74.5–92.5)</td>
<td>0.681</td>
</tr>
<tr>
<td>HDL-C, mmol/l</td>
<td>1.12 ± 0.07</td>
<td>1.73 ± 0.18</td>
<td>0.02</td>
</tr>
<tr>
<td>TGL, mmol/l</td>
<td>2.160 ± 0.393</td>
<td>0.820 ± 0.145</td>
<td>0.015</td>
</tr>
<tr>
<td>Calcium, mg/l</td>
<td>76.1 (71.3–82.7)</td>
<td>89.25 (85.73–94.05)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Magnesium, mg/l</td>
<td>16.56 ± 0.22</td>
<td>20.12 ± 1.25</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Zinc, mg/l</td>
<td>0.72 (0.62–1.04)</td>
<td>0.94 (0.76–1.18)</td>
<td>0.117</td>
</tr>
</tbody>
</table>

Notes: normally distributed characteristics are expressed as mean and standard deviation (SD) values. Non-normally distributed characteristics are expressed as medians (Q1–Q3). Means are compared by the Student’s t test and medians are compared by the Mann-Whitney U test. Nominal data are compared by the Chi-square test. FPG — fasting plasma glucose; HbA1c — glycated hemoglobin; BW — body weight; BMI — body mass index; WC — waist circumference; SBP — systolic blood pressure; DBP — diastolic blood pressure; HDL-C — high-density lipid cholesterol; TGL — triglycerides.

Results

Most of the examined patients (70.3 %) were diagnosed with DM. The rest of the patients had a disease duration of no more than 5 years. The level of fasting blood glucose of the patients ranged from 7.1 to 17.2 mmol/l and indicated the presence of DM. HbA1c levels in patients ranged from 7.1 to 11.2 %. According to age data, the majority of patients were in the age category of 50 years and older. Clinical picture fully corresponded to type 2 DM.

According to anthropometric data, the group of patients almost completely corresponded to the control group. Although a slight increase in body weight and waist circumference was registered already at the stage of initial DM, the level of triglycerides increased significantly and the level of high-density lipoproteins decreased (Table 1). There was also a tendency to increase systolic blood pressure.

The study of elements in patients showed that the content of calcium was in the range of 67.5–92.4 mg/l, the median was 76.1 mg/l. The first and third quartiles of the results are shown in Table 1. When compared with the control group, the serum level of total calcium was by 8.4 % lower compared to control patients (p < 0.011).

Magnesium interacts with calcium in many of its effects. In patients with DM, serum magnesium levels were also reduced by almost 8 %. Important meaning in the development of DM is given to zinc. In our studies, an abnormal distribution of the results of serum zinc content in patients with type 2 DM and the control group was observed. Therefore, we calculated the reliability for the abnormal series. Despite the visible distinction in medians, the difference was not significant. Analysis of individual results showed that the minimum value of zinc in patients was 0.518, while the maximum was 1.86 mg/l. Accordingly, healthy people have 0.676 and 1.405 mg/l.

It is worth noting that in patients with type 2 DM 44 % of samples had a value of about 0.6 mg/l, that is a lower reference index for zinc. Only 16.6 % of such samples were in control. It is possible that the level of reliability will change when the sample of studies and controls is increased.

Considering presence of changes in some parameters in patients with type 2 DM compared to controls, as well as a significant decrease in the serum level of total calcium and magnesium we conducted a correlation analysis between the determined indicators. We will not dwell in detail on all the obtained results. We will only give reliable correlations concerning blood elements and indicators. A positive and reliable correlation was established between the serum content of calcium and magnesium, and high-density lipids.

Table 2. Risks of type 2 DM depending on the serum level of essential elements (calcium, magnesium, zinc)

<table>
<thead>
<tr>
<th>Indicators</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca, mg/l</td>
<td>0.883</td>
<td>0.798</td>
<td>0.977</td>
</tr>
<tr>
<td>Mg, mg/l</td>
<td>0.543</td>
<td>0.343</td>
<td>0.859</td>
</tr>
<tr>
<td>Zn, mg/l</td>
<td>0.267</td>
<td>0.027</td>
<td>2.624</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>1.036</td>
<td>0.994</td>
<td>1.079</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>1.018</td>
<td>0.951</td>
<td>1.091</td>
</tr>
</tbody>
</table>

Notes: OR — odds ratio; CI — confidence interval; p < 0.05 — significance level.
A negative correlation was observed with the age of the subjects and fasting plasma glucose (FPG). Accordingly, magnesium showed a positive correlation with calcium and high-density lipids and a negative correlation with fasting blood glucose. The high negative correlation of zinc with the age of patients and systolic blood pressure is noteworthy. A high correlation between the concentration of calcium and magnesium in relation to FPG (mmol/l) indicates the important role of these elements in the pathogenesis of DM. To verify this position, we calculated the risk factors for these elements, as well as for one of the most important complications of type 2 diabetes, increased systolic and diastolic blood pressure (Table 2).

The obtained results indicate an increase in the risk of DM with a decrease in the concentration of calcium and magnesium in blood serum Regarding zinc, as well as systolic and diastolic blood pressure, we did not observe any significant changes. The insufficiently large sample did not allow us to calculate the risks for other indicators, which will be done in further studies.

Discussion
The study of macro- and microelements attracts the attention of specialists due to the fact that they are a constituent part of biological tissues and their exclusive regulatory participation in many processes and reactions in the body. In particular, calcium and magnesium are a component of bones, and due to their ionic form, they regulate the flow of ions and substances into various cells of the body.

Magnesium and zinc are cofactors of more than 300 enzymes and are part of them. An important aspect of the action of minerals is their influence on the immune system [18]. The study of indicators of the immune system in diabetes and minerals will help to approach their use more rationally [19].

Considerable attention is paid to these elements in the study of the DM pathogenesis [20]. Taking into account the obtained results and the value of these elements, it is necessary to the appointment of these elements in the DM management. However, ambiguous results are often obtained, which may be due to an insufficient assessment of the level of elements in the body of patients and the state of the disease [21].

In this regard, regional studies of elemental support, specific analysis, as well as objective assessment of the stage of the disease and the presence of its complications are necessary. Patients with the initial stage of the disease and in the absence of serious complications took part in our study. A decrease in the level of calcium and magnesium in the serum of patients with type 2 DM was established, which makes it possible to consider the need for the use of appropriate drugs and further study of their effect on the course of the disease.

Conclusions
A significant decrease in the level of calcium and magnesium in the blood of patients with type 2 diabetes mellitus in Ukraine was found.

There is a high correlation between calcium, magnesium and zinc levels in the blood of patients with type 2 DM. Significant risks of diabetes were revealed when the serum concentration of calcium and magnesium is decreased.

References
Оцінка рівня кальцію, магнію та цинку в сироватці крові хворих на цукровий діабет 2-го типу в українській популяції

Резюме. Актуальність. Дослідження присвячене вивченню відносного вмісту кальцію, магнію та цинку в сироватці крові хворих на ЦД2-го типу.

Мета: встановити рівень кальцію, магнію та цинку в сироватці крові хворих на ЦД 2-го типу.

Матеріали та методи. Відкрите контролюване дослідження включало 27 пацієнтів із ЦД2. Контрольну групу становили 12 осіб без ендокринних та соматичних захворювань. У 70 % хворих відзначені рівні кальцію, магнію та цинку в крові на рівні нижньої межі референтного значення.

Результати. Виявлена залежність між рівнем кальцію та магнію в крові хворих на ЦД2.

Висновки. Виявлено вірогідне зниження рівня кальцію та магнію в сироватці крові хворих на ЦД2.

Ключові слова: цукровий діабет 2-го типу, кальцій, магній, цинк.