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# Trace Elements, Magnesium, and the Efficacy of Antioxidant Systems in Children with Type 1 Diabetes Mellitus and in Their Siblings

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**A** – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

## Abstract

**Background.** Magnesium (Mg), selinium (Se), zinc (Zn), manganese (Mn), and copper (Cu) are involved in the mechanisms of antioxidant defense. Mn and Cu, which participate in the generation of reactive oxygen species (ROS), also have pro-oxidative properties.
**Objectives.** To evaluate the levels of Mg, Se, Zn, Mn, and Cu, as well as the effectiveness of antioxidant defense mechanisms in children with Type 1Diabetes Mellitus (T1DM) and in their siblings. The preliminary findings were originally reported in 2009 at the 35th annual conference of the International Society for Pediatric and Adolescent Diabetes (ISPAD) in Ljubljana, Slovenia.
**Material and Methods.** The study involved 87 children with T1DM, 2–19 years old, treated for T1DM for an average of 3.5 years. The sibling and control groups comprised 27 and 41 children, aged 4.5–16.5 years and 10.5–18 years respectively. The parameters named above were assessed in relation to metabolic compensation levels ($Hb_{A1C}$) and disease duration.
**Results.** Compared with the control group, T1DM children had lower plasma levels of Mg and Zn and higher levels of Cu; the siblings had lower levels of Zn; T1DM children had lower copper/zinc superoxide dismutase (CuZnSOD) activity; and both T1DM children and their siblings had higher catalase (CAT) activity and lower total antioxidant status (TAS) levels.
**Conclusions.** There may be a correlation between impaired antioxidant status and Mg and Zn deficiency and increased Cu levels in T1DM children. Oxidative stress in T1DM is accompanied by alterations in enzymatic activity and non-enzymatic mechanisms of antioxidant defense. The decreased TAS levels noted in T1DM patients may impair the effectiveness of non-enzymatic antioxidant systems. The increased CAT activity and unimpaired selenium-dependent glutathione peroxidase (Se-GSHPx) activity point indirectly to enhanced ROS generation in T1DM children. The impaired antioxidant defense found in the siblings of T1DM patients may indicate that genetic factors play a role (**Adv Clin Exp Med 2014, 23, 2, 259–268**).

**Key words:** type 1 diabetes mellitus, trace elements, magnesium, TAS, antioxidant enzymes.

Recent studies on the formation of reactive oxygen species (ROS) and oxidative stress have demonstrated that the hyperglycemia-induced process of peroxide generation in the mitochondrial chain of electron transport plays a key role in the activation of pathways responsible for the development of diabetic complications [1]. The activity of antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase (CAT), and the levels of small-molecule antioxidants, the serum activity of which is evaluated by determination of the so-called serum total antioxidant status (TAS), point to the role of oxidative stress in the pathomechanisms of diabetes mellitus and its complications [2].

Clinical manifestations of microvascular complications are rarely observed during the developmental age period; however, the biochemical

markers of oxidative stress associated with chronic hyperglycemia are prevalent in this age group [3]. Attempts to employ markers of oxidative stress as additional clinical manifestations to evaluate the metabolic status in diabetes or to predict the risk of developing late complications have been undertaken by numerous authors [4–7].

The elements magnesium (Mg), selenium (Se), zinc (Zn), manganese (Mn) and copper (Cu) are involved in the mechanisms of cellular antioxidant defense. Moreover, Cu and Mn, which participate in the generation of ROS, are known to possess pro-oxidative properties [8]. The participation of Mg in the mechanisms of antioxidant defense is associated with glutathione synthesis [9]. The antioxidant effect of Se is associated with its role as an indispensable component of the active SeGSH-Px center and thioredoxin reductase (TrxR). Furthermore, Se, along with vitamin E, inhibit lipid peroxidation [10, 11]. Zn exerts its antioxidant ability by stabilizing sulfhydryl groups in proteins and enzyme activity centers, protecting them against oxidation. One hypothesis for the mechanism of antioxidants suggests that an increased supply of these elements is beneficial because it leads to the stimulation of metallothionein synthesis, a protein containing a large number of thiol groups, which are effective in the reduction of ROS formation [8]. Zn ions, components of the CuZnSOD prosthetic group, participate in the enzymatic processes of cellular protection against ROS [11]. Mn is a co-factor in the key mitochondrial antioxidant enzyme, MnSOD [12]. Cu ions, which are also co-factors in CuZnSOD, participate in the reduction of $O_2^{\bullet-}$ to $H_2O$ [11]. Cu ions, alongside Fe ions, which are substrates in the Fenton reaction, participate in the generation of ROS. In this way, they participate in the process of oxidative damage, especially of DNA and low-density lipoprotein cholesterol (LDL-C) [8]. Copper also exhibits ferroxidase properties through its oxidization of Fe ions. It is capable of binding iron ions to transferrin and preventing the generation of ROS [13].

The aim of this study was to evaluate the levels of Mg, Se, Zn, Mn, and Cu as well as the effectiveness of antioxidant defense mechanisms in pubertal patients with type 1 diabetes mellitus (T1DM) and their siblings. The preliminary findings were originally reported in 2009 at the 35th annual conference of the International Society for Pediatric and Adolescent Diabetes (ISPAD) in Ljubljana, Slovenia.

## Material and Methods

The study involved 155 children and adolescents, who were divided into three groups. Group 1 included 87 children (49 boys and 38 girls) aged from 2 to 19 years (mean age 13.0 ± 4.0) being treated for T1DM (disease duration: 1 to 13 years) in the Clinic of Endocrinology and Diabetology of Developmental Age, Wroclaw Medical University, Wrocław, Poland. Group 2 included 27 children (11 boys, 16 girls) aged from 4.5 to 16.5 years (mean age 13.2 ± 3.7) who were siblings of the patients with T1DM. Group 3 was comprised of 41 healthy children (19 boys and 22 girls) aged from 10.5 to 18 years (mean age 14.8 ± 2.2) from non-diabetic families. The children had normal fasting glycemia and insulin findings, as well as normal body mass index (BMI: kg/m$^2$). The subjects' demographic data are presented in Table 1. The study protocol was approved by the Wroclaw Medical University Bioethical Committee (No. KB – 23/2006), and the study protocol was in accordance with the Declaration of Helsinki.

**Table 1.** The characteristics of the study groups

| | Patients with T1DM | Siblings of patients with T1DM | Controls |
|---|---|---|---|
| n | 87 | 27 | 41 |
| Gender (M/F) | 49/38 | 11/16 | 19/22 |
| Mean age (years) | 13.0 ± 4.0 | 13.2 ± 3.7 | 14.8 ± 2.2 |

In addition to total antioxidant serum status, the following parameters were evaluated in all the subjects: plasma levels of Mg, Mn, Cu, Zn and Se, and the activity of CuZn-SOD, CAT, and SeGSH-Px in erythrocyte hemolysate. In all the patients, glycated hemoglobin ($HbA_{1C}$) was measured and a lipidogram was performed (total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], LDL-C, and triglycerides [TG]). Blood samples were collected from the cubital vein in the morning, after fasting for 8 to 12 hrs. To evaluate blood serum, blood samples were collected and left for 15 min to form a clot at room temperature; then they were centrifuged at 3000 rpm for 15 min. To evaluate erythrocytes, blood samples were collected in tubes containing EDTA, and then centrifuged. Erythrocytes were washed three times with a cool 0.9% solution of NaCl, and then centrifuged. Red blood cells were suspended in 0.2 mL of 0.9% NaCl and frozen at –80°C. To evaluate the activity of antioxidant enzymes, an erythrocyte hemolysate was prepared by adding 0.15 mL of cool water to 0.025 mL of the investigated sample, which was kept on ice for 15 min. The activity of CuZn-

**Table 2.** Duration of the disease, $HbA_{1c}$, and lipid profiles in patients with T1DM, taking metabolic control into account

| | **Patients with T1DM (n = 87)** | **Patients with $HbA_{1C} \geq 8\%$ (n = 50)** | **Patients with $HbA_{1C} < 8\%$ (n = 37)** | **P[a]** |
|---|---|---|---|---|
| Duration years | 3.5 (0–13) | 5.0 (0–13) | 1.5 (0.17–9) | < 0.0001* |
| $HbA_{1C}$ | 8.5% (5.7–15.1) | 9.0% (8.0–15.1) | 7.1% (5.7–7.9) | < 0.0001* |
| Cholesterol mg/dL | 175 (108–293) | 174 (126–293) | 176 (108–245) | 0.806 |
| HDL-C mg/dL | 64.3 (35.3–102.0) | 64.4 (35.3–102.0) | 64.0 (41.2–100.0) | 0.957 |
| LDL-C mg/dL | 97.0 (68.4–140.0) | 97.3 (68.4–140.0) | 96.2 (75.8–118.0) | 0.873 |
| TG mg/dL | 90.9 (30.5–324.0) | 97.2 (42.5–324.0) | 79.4 (30.5–187.0) | 0.143 |
| Cholesterol/HDL-C | 2.83 (1.90–4.74) | 2.84 (1.90–4.74) | 2.79 (1.93–3.94) | 0.813 |

* statistically significant values.
[a] patients with T1DM vs. controls.

SOD in blood erythrocytes was assessed by means of a modified RANSOD protocol (RANDOX Laboratories Ltd., Great Britain).

The activity of CAT in blood erythrocytes was assessed according to the procedure described by Bartosz [14]. The activity of SeGSH-Px in erythrocytes was evaluated by the RANSEL protocol (RANDOX Laboratories Ltd., Great Britain). TAS in serum was assessed via a kit manufactured by Randox Laboratories Ltd. (Crumlin, Great Britain). The result was expressed in mmol/L of Trolox, a reference antioxidant (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), which is a vitamin E derivative. The plasma levels of the elements were determined via flame spectrophotometry using a SOLAAR M6 Atomic Absorption Spectrophotometer (manufactured by Thermo Elemental, Great Britain): Mg at λ wavelength = 285.2 nm; Cu at λ wavelength = 213.9 nm; Mg at λ wavelength = 324.8 nm in an air-acetylene flame using deuterium background correction; Mn at λ wavelength = 279.5 nm; and Se at λ wavelength = 196.0 nm using an electrographite cuvette with Zeman's correction. In this method, the modification of the matrix was achieved by the addition of a nickel nitrate modifier.

A distribution analysis of the investigated parameters was performed using the D'Agostino-Pearson normality test. Variables with normal distribution were presented as mean values (TAS, CAT); when there was a lack of normal distribution, the findings were presented as median values (CuZnSOD, SeGSH-Px). The investigated groups were compared, taking into account their distribution and size. Variables with normal distribution were analyzed with the ANOVA parametric test to evaluate the mean from many groups, the *t*-test to compare the mean between two groups, and Pearson's test to analyze correlations. Variables without normal distribution were analyzed by means of nonparametric tests: the Kruskal-Wallis test to compare the median among many groups, the Mann-Whitney *U* test to compare the median between two groups, and Spearman's rank test to analyze correlations. The analysis of prevalence was performed by means of the chi-square test. The mean and median values were presented along with their 95% confidence interval. All the analyses were assumed statistically significant at $p < 0.05$. All the statistical analyses were conducted with MedCalc® Version 9.2.1.0 software.

# Results

## Plasma Levels of the Elements Mg, Mn, Cu, Zn, and Se

In patients with T1DM, their siblings and the control subjects, Mn and Se levels were normal and there were no differences in these elements among the three groups. The levels of Mg and Zn in children with T1DM were significantly lower compared with the controls ($p = 0.0009$ and $p = 0.046$, respectively). Moreover, Zn levels were significantly lower in the sibling group than in the control group ($p = 0.040$). The level of Cu in children with T1DM was significantly higher compared with the control and sibling groups ($p = 0.0003$ and $p = 0.0007$, respectively) (Table 3). The analysis revealed a tendency for a negative correlation to appear between the levels of Mg, Mn and Cu and the age of patients with T1DM ($r = -0.21$, $p = 0.0521$; $r = -0.21$, $p = 0.057$; $r = -0.42$, $p < 0.01$). There was also a tendency toward a negative correlation with the level of Mg and duration of the disease ($r = -0.20$, $p = 0.059$) (Table 4). Among all the elements investigated, only Mg correlated with HDL-C ($r = 0.33$, $p = 0.043$). There was also a tendency toward a positive correlation between the Cu/Zn ratio and HDL-C ($r = 0.31$, $p = 0.053$) and Cu and TC ($r = 0.26$, $p = 0.052$) (Table 5). Among children with T1DM, there was a positive correlation among the levels of Mn, Zn and

**Table 3.** Comparison of the levels of elements in patients with T1DM, their siblings and the controls

| | **Patients with T1DM (n = 87)** | **Siblings of patients with T1DM (n = 27)** | **Controls (n = 41)** | **p** |
|---|---|---|---|---|
| Mg µg/mL | 19.27 (18.93–19.62) | 20.18 (19.48–20.88) | 20.54 (19.82–21.25) | *0.0009[a]<br>*0.015[b]<br>0.461[c] |
| Se µg/L | 58.4 (55.0–63.09) | 53.45 (46.09–65.21) | 53.3 (45.78–70.17) | 0.441[a]<br>0.487[b]<br>0.991[c] |
| Zn µg% | 88.35 (82.25–91.76) | 87.20 (77.60–93.94) | 97.00 (87.64–103.00) | *0.046[a]<br>0.601[b]<br>*0.040[c] |
| Cu µg% | 126.19 (122.27–130.23) | 111.79 (104.33–119.79) | 110.55 (102.19–119.60) | *0.0003[a]<br>*0.0007[b]<br>0.827[c] |
| Mn µg/L | 1.58 (1.41–1.77) | 1.29 (1.03–1.62) | 1.55 (1.38–1.74) | 0.832[a]<br>0.103[b]<br>0.145[c] |

Mean and median values are presented with a 95% confidence level.
[a] patients with T1DM vs. controls, [b] patients with T1DM vs. siblings, [c] siblings vs. controls.
* statistical significance at $p < 0.05$.

**Table 4.** Correlations between the element serum levels and gender, age, BMI, disease duration and $HbA_{1C}$ in patients with T1DM

| | **Mg** | **Mn** | **Cu** | **Zn** | **Se** |
|---|---|---|---|---|---|
| Gender | p = 0.773 | p = 0.054 | p = 0.124 | p = 0.913 | p = 0.220 |
| Age | r = –0.21,<br>p = 0.052** | r = –0.21<br>p = 0.057** | r = –0.42<br>p < 0.01* | r = 0.02<br>p = 0.858 | r = 0.01<br>p = 0.893 |
| BMI | p = 0.093 | p = 0.778 | p = 0.779 | p = 0.713 | p = 0.508 |
| Time | r = –0.20<br>p = 0.058** | r = 0.01<br>p = 0.916 | r = –0.03<br>p = 0.804 | r = 0.02<br>p = 0.867 | r = 0.06<br>p = 0.567 |
| $HbA_{1C}$ | r = –0.001<br>p = 0.673 | r = –0.04<br>p = 0.698 | r = 0.01<br>p = 0.897 | r = –0.07<br>p = 0.541 | r = 0.20<br>p = 0.069 |

* statistical significance at $p < 0.05$.
** tendency to statistical significance at $0.05 < p < 0.1$.

TAS ($r = 0.22$, $p = 0.04$; $r = 0.38$, $p = 0.0004$), and a negative correlation between the Cu/Zn ratio and TAS ($r = –0.38$, $p = 0.0004$) (Table 6).

## The Activity of Antioxidant Enzymes in Erythrocytes and TAS

The activity of CuZnSOD in patients with T1DM was significantly lower than in the controls ($p < 0.0001$). There was no significant difference in SeGSH-Px activity among the 3 groups. The level of CAT significantly increased in children with T1DM compared with the controls ($p < 0.0001$). Patients with T1DM showed a positive correlation between the level of Se and the amount and activity of GSH-Px co-factor ($r = 0.39$, $p = 0.046$) (Table 6). The siblings of patients with T1DM revealed similar enzymatic activity; the level of CuZnSOD was significantly lower ($p < 0.0001$), the level of CAT was significantly higher ($p = 0.0006$), and there was no difference in the level of SeGSH-Px compared with the controls (Table 7). TAS levels were significantly lower in children with T1DM compared with the controls ($p < 0.0001$) and their siblings ($p < 0.0001$). No correlation was found between the activity of antioxidant enzymes and the gender or age of the subjects, duration of the disease, and $HbA_{1C}$ (Table 8).

**Table 5.** Correlations between lipid profile parameters and element serum levels in patients with T1DM

| | TC | TG | HDL–C | LDL–C | TC/HDL–C |
|---|---|---|---|---|---|
| Mg | r = 0.07<br>p = 0.616 | r = –0.09<br>p = 0.517 | r = 0.33<br>p = 0.043* | r = –0.06<br>p = 0.714 | r = –0.11<br>p = 0.502 |
| Mn | r = –0.11<br>p = 0.399 | r = –0.10<br>p = 0.462 | r = –0.05<br>p = 0.749 | r = –0.02<br>p = 0.904 | r = 0.06<br>p = 0.729 |
| Cu | r = 0.26<br>p = 0.052** | r = 0.01<br>p = 0.962 | r = 0.29<br>p = 0.067 | r = 0.03<br>p = 0.867 | r = –0.03<br>p = 0.867 |
| Zn | r = 0.23<br>p = 0.086 | r = 0.22<br>p = 0.113 | r = –0.10<br>p = 0.551 | r = 0.03<br>p = 0.837 | r = 0.15<br>p = 0.344 |
| Cu/Zn | r = 0.06<br>p = 0.663 | r = –0.25<br>p = 0.071 | r = 0.31<br>p = 0.053** | r = –0.01<br>p = 0.943 | r = –0.16<br>p = 0.327 |
| Se | r = –0.05<br>p = 0.723 | r = –0.07<br>p = 0.588 | r = –0.12<br>p = 0.453 | r = –0.02<br>p = 0.890 | r = 0.20<br>p = 0.223 |
| TAS | r = –0.11<br>p = 0.393 | r = 0.02<br>p = 0.886 | r = –0.19<br>p = 0.229 | r = –0.152<br>p = 0.35 | r = 0.137<br>p = 0.400 |

* statistical significance at $p < 0.05$.
** tendency to statistical significance at $0.05 < p < 0.1$.

**Table 6.** Correlations between element serum levels and antioxidant enzyme activity in patients with T1DM

| | CuZnSOD | CAT | SeGSHPx | TAS |
|---|---|---|---|---|
| Mg | r = –0.15<br>p = 0.456 | r = –0.29<br>p = 0.144 | r = 0.05<br>p = 0.806 | r = 0.09<br>p = 0.399 |
| Mn | r = 0.06<br>p = 0.751 | r = –0.04<br>p = 0.858 | r = –0.06<br>p = 0.776 | r = 0.22<br>p = 0.04* |
| Cu | r = –0.01<br>p = 0.953 | r = –0.34<br>p = 0.079 | r = 0.10<br>p = 0.604 | r = –0.09<br>p = 0.428 |
| Zn | r = 0.14<br>p = 0.482 | r = –0.11<br>p = 0.557 | r = 0.09<br>p = 0.659 | r = 0.38<br>p = 0.0004* |
| Cu/Zn | r = –0.05<br>p = 0.655 | r = –0.16<br>p = 0.419 | r = 0.15<br>p = 0.449 | r = –0.38<br>p = 0.0004* |
| Se | r = 0.28<br>p = 0.153 | r = –0.09<br>p = 0.658 | r = 0.3<br>p = 0.046* | r = –0.07<br>p = 0.513 |

* statistical significance at $p < 0.05$.

# Discussion

Increasing attention has been given to the role of certain elements in the pathogenesis of diabetes mellitus and in the progression of its complications [15]. This study evaluated the levels of elements that participate in various mechanisms of antioxidant defense. Kruse-Jarres et al. [16] compared the levels of Zn, Cu, Cr, and Se in complete blood, plasma, and in blood morphotic elements (erythrocytes, platelets, neutrophils) in children with T1DM and in patients with type 2 diabetes mellitus (T2DM). They measured higher Cu levels in all the investigated fractions of patients with T1DM. The level of Zn was higher only in complete blood and in erythrocytes, and the levels were lower in blood plasma, platelets and neutrophils. A Zn deficiency was more pronounced in poorly controlled T1DM (> 9% $HbA_{1C}$), and this correlated with the severity of hyperglycemia. According to these researchers, this deficit might reflect a renal loss of the element and its passage from the plasma to the erythrocytes.

Evidence from epidemiologic studies demonstrated an association between a Mg-rich diet and decreased incidence of T1DM and its complications [17].

According to other researchers, the condition

**Table 7.** Comparison of CuZnSOD, SeGSH–Px, and CAT and TAS activity in patients with T1DM, their siblings and the controls

| | Patients with T1DM | Siblings of patients with T1DM | Controls | P |
|---|---|---|---|---|
| CuZnSOD U/mg Hb | 0.945 (0.763–1.060) | 0.770 (0.523–1.860) | 2.004 (1.499–3.416) | *< 0.0001[a] 0.6[b] *0.001[c] |
| SeGSH-Px U/mg Hb | 41.22 (35.14–48.37) | 36.22 (13.21–45.92) | 35.57 (23.22–51.62) | 0.333[a] 0.289[b] 0.704[c] |
| CAT U/mg Hb | 0.888 (0.846–0.930) | 0.916 (0.776–1.005) | 0.710 (0.661–0.759) | *< 0.0001[a] 0.651[b] *0.0006[c] |
| TAS mmol/L | 0.894 (0.854–0.935) | 0.932 (0.865–1.005) | 1.170 (1.101–1.243) | *< 0.0001[a] 0.360[b] *< 0.0001[c] |

Mean and median values are presented with a 95% confidence level.
[a] patients with T1DM vs. controls, [b] patients with T1DM vs. siblings, [c] siblings vs. controls.
* statistical significance at $p < 0.05$.

**Table 8.** Correlations between CuZnSOD, SeGSH–Px, CAT and TAS activity and gender, age, disease duration and $HbA_{1C}$ in patients with T1DM. Statistical significance at $p < 0.05$

| | CuZnSOD | SeGSH–Px | CAT | TAS |
|---|---|---|---|---|
| Gender | p = 0.803 | p = 0.204 | p = 0.115 | p = 0.109 |
| Age | r = –0.018 p = 0.947 | r = 0.003 p = 0.981 | r = –0.04 p = 0.713 | r = 0.042 p = 0.960 |
| Time | r = –0.17 p = 0.127 | r = 0.34 p = 0.765 | r = 0.02 p = 0.855 | r = 0.037 p = 0.731 |
| $Hb_{A1C}$ | r = –0.17 p = 0.159 | r = 0.08 p = 0.495 | r = –0.0 p = 0.504 | r = –0.120 p = 0.270 |

is due to increased urinary excretion of Mg [18], and/or dietary deficiency and decreased absorption of Mg [19–21]. McNair et al. [22] explained low plasma levels of Mg, urinary excretion of Mg and fasting glycemia as being caused by decreased urethral reabsorption of this element in the presence of hyperglycemia. Mg supplementation in deficient patients with T1DM improved their insulin sensitivity and decreased atherogenic lipid fractions, while Mg supplementation reduced the risk of cardiovascular complications in both types of diabetes [19, 20].

In the current study, the patients with T1DM had significantly lower levels of Mg compared with the controls, but metabolic control and the duration of the disease were not found to affect Mg levels. Significantly lower levels of Mg and significantly higher blood arginase activity in children with T1DM were demonstrated by Bjelakovic et al. [23] This could be a consequence of reduced insulin action and increased protein catabolic processes in these pathophysiologic conditions. Guerrero-Romero et al. [24] and Sales et al. [19] demonstrated that in patients with T2DM hypomagnesemia, there was a negative correlation with low levels of HDL-C and the concentrations of albumin in transferrin, regardless of glycemia. A negative correlation between the levels of Mg and HDL-C was also revealed in the present study. Atabek et al. [25] noted a relationship between Mg deficiency and the early onset of atherosclerosis, independently of $HbA_{1C}$ level and lipid profile. Mg deficiency correlated significantly with the intima-media thickness. The element Se was decreased in the plasma of patients with diabetes, and – consistent with the findings of Kruse-Jarres et al. [16] – the current study did not demonstrate any significant difference in the levels of Se between children with T1DM and the controls. However, a decreased level of Se in patients with diabetes, regardless of their gender and age, was reported in a study by Nawarro-Alarcon et al. [26], and a negative correlation

with $HbA_{1C}$ in a study by Ruiz et al. [27] The results of the current study did not demonstrate that Se had any effect on metabolic control or the duration of diabetes. The level of Se correlated positively with the activity of erythrocyte GSH-Px, a Se-dependent enzyme. Other studies point to an increased level of Se in children with T1DM compared with healthy children, which correlated positively with the level of lipoproteins, but not with GSH-Px activity [28, 29].

*In vitro* studies have demonstrated that the peroxidation of lipid molecules induced by Cu and Fe ions was significantly enhanced in the presence of glucose [30]. Patients with either type of diabetes developed higher levels of Cu in extracellular spaces. These higher levels, along with the presence of ceruloplasmin, play a role in the oxidative modification of LDL-C particles [21, 31, 32]. Awadallah et al. [33] explained that increased levels of Cu and ceruloplasmin in the sera of patients with cardiovascular disease was caused by intensification of the inflammatory process, a primary factor in atherogenesis. According to Shukla et al. [31], an increased level of Cu in cardiovascular disease, as well as in diabetes, is associated with the effect of ROS, which block the attachment of Cu to ceruloplasmin. Cu ions are necessary to enable proper activity of C cytochrome, antioxidant enzymes such as dismutase, metallothionein and other cellular oxidases. The activity of erythrocyte CuZnSOD is considered a more reliable marker for Cu status than the serum level of Cu [34]. In the current study, the level of Cu in children with T1DM was higher than in the control group, but no correlation of its level with the activity of CuZnSOD was found.

Zn is a ROS-formation antagonist, and acts as an antioxidant because it protects sulfhydryl groups against oxidation. Zn, together with Cu, is a cofactor of CuZnSOD, a ROS-scavenging enzyme [15]. The present study revealed a Zn deficiency in T1DM; similar observations were reported by Rohn et al. [35], who emphasized the significance of Zn supplementation in patients with diabetes. Ruíz et al. [36] did not note any differences in the levels of Zn between patients with T1DM and the controls. In the present study, a positive correlation was observed between the levels of Zn and TAS in children with T1DM, which confirms the antioxidative role of this element.

A high level of Mn accompanies intense lipid peroxidation in patients with diabetes, increasing the likelihood that they will develop cardiovascular disease [37]. In the present study groups, no significant differences in Mn levels were observed; however, there was a positive correlation between Mn and TAS in children with T1DM.

A significant role in the etiology of chronic diseases is attributed to the Cu/Zn ratio. This ratio may be considered to be an oxidative status exponent, as an increased Cu/Zn ratio in aging processes and in senile degenerative diseases correlates with the severity of oxidative stress [38]. The current study revealed a positive correlation between Cu/Zn and TAS. Mezzetti et al. [39] reported a positive correlation between Cu/Zn and the level of TGC; however, in the present study, there was a weak positive correlation between the Cu/Zn ratio and HDL-C.

## Intra-Erythrocyte Antioxidant Enzymes and TAS

Gene expression and the activity of antioxidant enzymes, such as CuZnSOD, ZnSOD, GSH-Px and CAT, occur at a lower level in pancreatic islet cells than in other tissues in the organism. This results in an increased susceptibility to ROS-inflicted damage [39]. Tiedge et al. [40] discovered that the cytoplasmic CuZnSOD and mitochondrial MnSOD genes exhibit only 30% to 40% expression in the liver compared with the pancreas, and the expression of GSH-Px in the pancreas was merely 15% of that which normally occurs in the liver, while CAT gene expression was absent in the pancreas. An increase in the activity of GSH-Px in hyperglycemia protects beta cells against oxidative damage [41]. Antioxidant activity may be evaluated by measuring the activity of individual free-radical scavenging enzymes. Diseases accompanied by increased ROS synthesis are associated with an initial compensatory increase in antioxidant activity, which decreases during prolonged oxidative stress. This reflects the depletion of the systemic pool of enzymes and antioxidants. Zivić et al. [42] reported a significant increase in CAT activity during different phases of T1DM in children, that is, at the beginning of diabetes, during the remission period, and later in the chronic course of the disease, compared with a control group. The highest CAT activity occurred during the early course of the disease, and was followed by a linear decrease, with the lowest activity during the chronic course. CAT activity was in direct correlation with $HbA_{1C}$ and was inversely correlated with C-peptide. The current study showed a significantly lower CuZnSOD level, a lack of any difference in SeGSH-Px activity, and significant CAT activity in patients with T1DM compared with the controls.

Data from Chiarelli et al. [43] revealed an increase in the activity of CuZn-SOD, CAT and GSH-Px in the skin fibroblasts of T1DM patients without complications, while only increased CuZn-SOD activity was seen in the group with diabetic

microangiopathy. Increased CuZn-SOD activity and decreased GSH-Px in children and adolescents in the early stages of T1DM and in patients with poor metabolic control have been reported by other authors [44, 45]. Domínguez et al. [44] also confirmed a negative correlation between the level of $HbA_{1C}$ and GSH-Px and CuZnSOD activity. Elhadd et al. [46] suggested that puberty may negatively modulate the function of the endothelium and the mechanisms of antioxidant defense. These researchers demonstrated that the endothelial function markers, E-selectin and the ICAM-1 adhesive molecule, were significantly increased in pubertal T1DM children when compared with a group of young adults, while the activity of SOD was significantly higher in prepubertal children compared with adolescents and young adults. SOD activity increased as a result of intense oxidative stress, and it decreased in older children due to the depletion of its activity as a result of chronic oxidative stress. The activity of erythrocyte CuZnSOD depends on two elements: Cu and Zn. Per Shukla et al. [31] suggested that in many diseases associated with chronic oxidative stress, including diabetes, ROS impair the function of CuZn -SOD by replacing the intracellular Cu ions found in protein compounds, eg, in metallothionein and also in SOD. Another explanation for decreased CuZnSOD activity is a Zn deficiency in the erythrocytes of patients with diabetes [26].

The available literature contains few reports on the antioxidant status of the relatives of patients with T1DM. A study by Varvarovská et al. [47] revealed significantly less activity of GSH-Px in the siblings of children with T1DM, along with lower SOD activity, unchanged GSH-Px, and significantly increased CAT compared with the controls. In the current study, the activity of enzymes in children with T1DM and in their siblings was similar. The results showed a significant decrease in TAS in children with T1DM, regardless of the duration of the disease and metabolic control. This observation is consistent with the findings of other researchers [48, 49]. Decreased levels of TAS, which result from the depletion of endogenous antioxidants, confirm the presence of oxidative stress in children with T1DM. Astaneie et al. [48] demonstrated a decreased level of TAS in children with T1DM, decreased TAS, and an unchanged level of lipid peroxidation products (malondialdehyd, MDA). Those researchers explained that sufficient antioxidant defense in this period prevents effective peroxidation processes. The present study did not reveal any correlation between TAS levels and the age of patients, the duration of their disease, $HbA_{1C}$ or BMI. Similar results were obtained by Marra et al. [49]; however, apart from decreased levels of TAS in patients with T1DM, Valabhji et al. [50] and Vantyghem et al. [51] found a negative correlation between TAS and $HbA_{1C}$ levels and duration of the disease, and no correlation with BMI and lipid profile. In the present study, TAS did not correlate with the components of the lipid profile. Data from Reis et al. [52] showed that in comparison with healthy individuals, patients with T1DM exhibited an increase in ROS generation, while plasma antioxidant status remained unaltered. Matteucci et al. [53] demonstrated a disturbed antioxidant status in first-degree relatives of patients with T1DM, which may have been caused by a general pro-oxidative background resulting from an impaired erythrocyte redox system. In the present study, TAS in the siblings of patients with T1DM was significantly lower than in the controls, and the amount was comparable to that of patients with diabetes. Similar findings were presented by Varvarovská et al. [47]. In conclusion, the results of the current study may suggest a genetic background for metabolic disturbances; however, this hypothesis requires extensive studies of first-degree relatives of patients with T1DM.

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