Neuroendocrinology Letters Volume 43 No. 5 2022
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

Do trace elements influence the course of newly diagnosed type 1 diabetes mellitus?

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Submitted: 2022-03-07 Accepted: 2022-11-12 Published online: 2022-11-12

Key words: **children; type 1 diabetes mellitus; trace elements; selenium; zinc; copper; arsenic**

Neuroendocrinol Lett 2022; **43**(5):247–256 **PMID:** 36584401 NEL430522A02

Abstract

The etiology of type 1 diabetes mellitus (DM1) is not fully understood. Some studies indicate an excess or deficiency of certain trace elements may affect glucose and insulin metabolism. This study aimed to assess the concentrations of trace elements in children with newly diagnosed DM1. The study group comprised 35 children aged 3–17 years (mean, 8.83±3.55 years). Serum concentrations of selenium, zinc, copper, and arsenic were determined at the time of diagnosis, after ~2 weeks (during insulin treatment), and after 6 months. No trace element deficiency was observed. Selenium levels were increased at all time points (77.61±14.03 μg/l; 70.42±11.04 μg/l; 75.79±12.89 μg/l). Arsenic levels were increased at the time of discharge (0.30±0.24 µg/l) and upon 6 months control visit (0.67±1.98 μg/l) for DM1. Copper levels were elevated at the time of diagnosis (1333±244 μg/l). No significant differences were observed in zinc concentrations between study and control group or between time points. Trace elements in the environment, especially selenium, may increase the incidence of DM1, although further research is required to confirm this association.

Abbreviations:

ANOVA	- analysis of variance
Anti-ZnT8	- zinc transporter 8 antibodies
DKA	- diabetes ketoacidosis
DM1	- type 1 diabetes mellitus
ELISA	- enzyme-linked immunosorbent assay
GADA	- anti-glutaminic acid decarboxylase antibodies
HbA1c	- glycated hemoglobin, hemoglobin A1c
HDL	- high density lipoprotein
IA/IAA	- anti-insulin autoantibodies
IA2A	- insulinoma- associated protein2 antibodies/ islet antigen2
IASP	- Islet Autoantibody Standardization Program
ICA	- islet cell anibody
LDL	- low density lipoprotein
NS	- non-significant
p	- probability value
pH	- inverse logarithm of the concetration of hydrogen ions
r	- correlation coefficient
SD	- standard deviation
SE	- standard error
TC	- total cholesterol
TG	- triacyloglycerol

INTRODUCTION

DM1 is an autoimmune disease that mainly affects children and young adults. The etiology of type 1 diabetes mellitus (DM1) is multifactorial; however, the precise influence of genetic predisposition, environmental factors, and β-cell damage is not entirely understood (Mayer-Davis *et al.* 2018). For example, some studies have shown that a deficiency or excess of some trace elements (e.g., selenium, zinc, copper, and arsenic) may affect glucose metabolism (Castillo-Durán & Cassorla 1999; Obeid *et al.* 2008; Alfawaz *et al.* 2019). However, the influence of various trace elements on the development and course of DM1 has not been well studied.

Selenium (Se) has antioxidant properties (Peruzzu *et al.* 2015) and plays important roles in oxidative stress, insulin signaling, insulin resistance, and carbohydrate and lipid metabolism (Wei *et al.* 2015; Solovyev *et al.* 2019). However, clinical reports on the influence of selenium levels in patients with diabetes remain controversial (Kljai & Runje 2001).

Zinc (Zn), a trace element that participates in over 300 enzymatic reactions (Obeid *et al.* 2008; Dubey *et al.* 2020), may also contribute to the pathogenesis of DM1. Zinc plays an essential role in the synthesis, storage, and secretion of insulin (Castillo-Durán & Cassorla 1999; Kawasaki 2012; Singh Malik *et al.* 2020). In particular, the SLC30A8 zinc transporter (ZnT8) facilitates zinc transport into cells, which promotes the maturation and crystallization of insulin (Gaither & Eide 2001; Davidson *et al.* 2014). Moreover, ZnT8 antibodies, which were discovered relatively recently, are detected in 60–80% of patients with DM1 (Wenzlau *et al.* 2007; Kawasaki 2012; Adulcikas *et al.* 2019). Indeed, zinc deficiency may occur in diabetes, and its supplementation can inhibit the development of experimental DM1 in mice (Kawasaki 2012). Therefore, low zinc levels may influence the development of DM1.

Copper (Cu) has been shown to contribute to the pathogenesis of diabetes, primarily due to oxidative mechanisms (Lowe *et al.* 2017; Samadi *et al.* 2020). Copper is a key component of many enzymes that catalyze oxidation-reduction reactions and are involved in transport, production, and detoxification (Obeid *et al.* 2008). Moreover, many studies have reported elevated copper concentrations in patients with diabetes, in whom complications such as retinopathy, microvascular disease, or hypertension occur (Walter *et al.* 1991). However, copper concentrations in those with newly diagnosed DM1 have not been routinely studied.

Studies conducted in arsenic (As) endemic areas (e.g., Taiwan, Bangladesh, and Mexico) showed that chronic exposure to high concentrations of arsenic (≥100 μg/l) was associated with high levels of glycated hemoglobin (HbA1c) and increased risk of diabetes (Sabbioni *et al.* 1991; Jensen & Hansen 1998; Aposhian & Aposhian 2006; Navas-Acien *et al.* 2006; Coronado-González *et al.* 2007; Goldman 2019). Contaminated water and food are the biggest sources of exposure to inorganic arsenic (Sabbioni *et al.* 1991), which is highly toxic to humans (Aposhian & Aposhian 2006; Goldman 2019). In the case of diabetes, exposure to arsenic can inhibit insulin-dependent glucose uptake and impair insulin secretion (Rhee *et al.* 2013), likely due to expression changes in genes associated with insulin resistance in β-cells (Longnecker & Daniels 2001; Díaz-Villaseñor *et al.* 2006). However, this relationship is not fully understood, and the results remain controversial (Ruiz-Navarro *et al.* 1998; Longnecker & Daniels 2001; Zierold *et al.* 2004; Lamm *et al.* 2006; Navas-Acien *et al.* 2006; Wang *et al.* 2007; Chen *et al.* 2010).

This study aimed to assess the concentrations of trace elements (Se, Zn, Cu, As) in children with newly diagnosed DM1 and their association with the disease course.

MATERIALS AND METHODS

The study group comprised 35 children (28 boys and 7 girls) aged 3–17 years (mean, 8.83±3.55 years) with newly diagnosed DM1. Patients were enrolled from September 2017 to September 2018. Before entering the study, parents received written and oral information about the methodology and the purpose of the study, voluntary accession, and the possibility of withdrawal at any stage of the study, without giving any reason.

The control group comprised 31 children (26 boys and 5 girls) aged 2–16 years (mean, 8.94±3.64 years) without metabolic disorders. These children were admitted to the Department of Pediatric Surgery for small surgical procedures, for instance: phimosis, gynecomastia, excision/ removal of skin lesion or hernia. All children from both groups where did not suffer from any chronic diseases. The study and control groups did not differ significantly in terms of age and gender (p=0.8988). The study received the approval of the Bioethics Committee of the Pomeranian Medical University in Szczecin (no. KB-0012/34/17).

The inclusion criteria were as follows: diabetes diagnosed based on a random blood glucose level of >200 mg/dl and typical clinical symptoms according "The 2018 Guidelines on the management of diabetic patients. A position of Diabetes Poland" (Diabetology 2018), and parent/legal guardian written consent. The exclusion criteria included: lack of written consent, general severe condition which required intensive care unit (pH≤7,0), prior oncological treatment, a history of antibiotic treatment, insulin therapy prior to study or taking vitamin/mineral supplementations.

Flow chart of the study is presented in figure 1. The clinical and biochemical characteristic of the study and control group is presented in table 1.

All subjects underwent a dietary consultation with an assessment of their eating habits. Subsequently, their diets have been corrected and recommendations were issued. They included a balanced diet with low

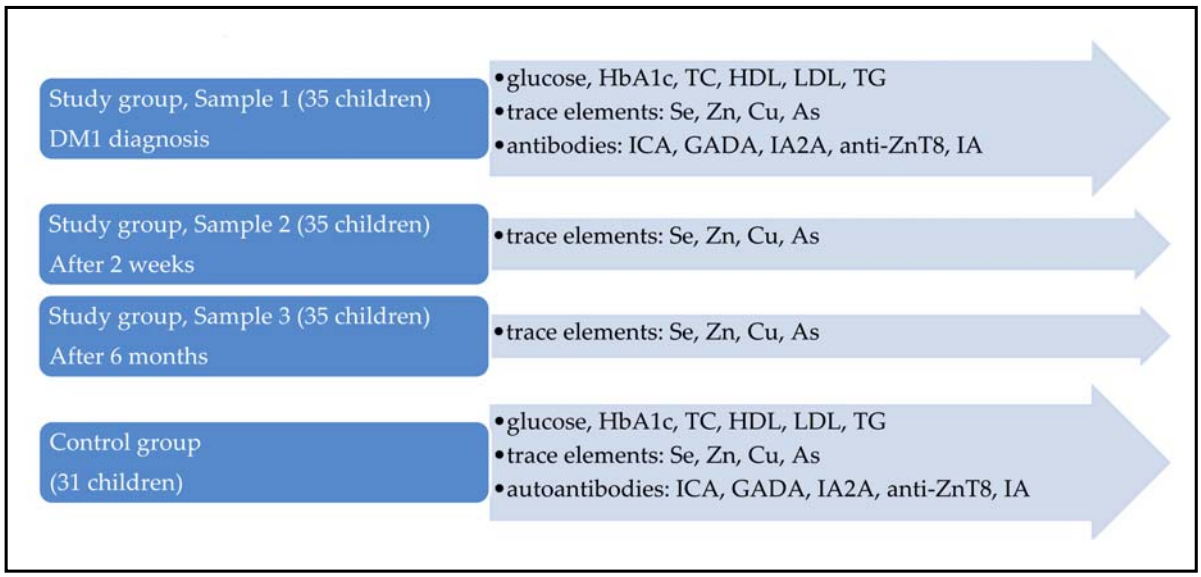

Fig. 1. Flow chart of the study
Anti-ZnT8 – zinc transporter 8 antibodies; GADA – anti-glutaminic acid decarboxylase antibodies; HbA1c – glycated hemoglobin; HDL – high density lipoprotein; IA2A – insulinoma- associated protein2 antibodies/ islet antigen2; ICA – islet cell anibody; LDL – low density lipoprotein; TC – total cholesterol; TG – triacyloglycerol.

glycaemic index and a method of quantifying carbohydrate (CHO) intake. Nutrition rules according to the norm for children with DM1 from 2018 (Diabetology 2018) include: carbohydrates accounting for 45-65% of energy, fats for 30% of energy, proteins for 10-20% of energy. The recommendations took into account the degree of physical activity of the child and were modified to their current nutritional status. The recommended diet did not contain seafood. The test group was asked not to use dietary supplements.

Serum sample 1 was collected immediately after admission to hospital, at the time of diagnosis. Serum sample 2 was collected before leaving hospital, after achieving metabolic control and initiation of insulin therapy. The average time interval between consecutive samples was 9.3±2.8 days. Serum sample 3 was collected during a one-day clinic visit, and the average time from the first hospitalization was 8.31±1.94 months.

In the study group (35 children) mean pH was 7.34 ± 0.11 and level bicarbonate was 16.11±7.35 mmol/l. Diabetes ketoacidosis (DKA) was present in 12 children (34.29%): mild (pH <7.3 and/or bicarbonate <15 mmol/l) in 3 children (8.57%), moderate (pH<7.2 and/or bicarbonate <10 mmol/l) in 5 children (14.29%) and severe (pH <7.1 and/or bicarbonate <5 mmol/l) in 4 children (11.43%). No child was diagnosed with any other infection. The children did not take any medication that could affect the carbohydrate metabolism.

Sample collection and storage

Sera were collected with the Sarstedt Monovette system (Sartedt, Germany) using Serum Z/7.5 ml tubes by a standard antecubital venipuncture. Collected sera were incubated at room temperature for at least 30 min to clot (but for no longer than 2 h) before being centrifuged at 1300 G for 12 min. Sera were then transferred into cryovials and stored at -80°C until further analysis. On the day of analysis, sera were thawed, vortexed, and centrifuged at 5000 G for 5 min before trace element determination.

Trace metal measurements

Determination of arsenic (75As), zinc (66Zn), copper (65Cu), and selenium (80Se) levels was performed using an ICP mass spectrometer ELAN DRC-e (PerkinElmer). Before each analytical run, the instrument was tuned to achieve the manufacturers' criteria. Oxygen was used as a reaction gas. Technical details are available on request.

The spectrometer was calibrated using an external calibration technique. Calibration standards were prepared fresh daily, from 10 µg/ml Multi-Element Calibration Standard 3 (PerkinElmer, USA) by diluting with the blank reagent to a final concentration of 0.48, 0.99, and 1.98 μg/l for As determination and to 1, 2, 5, 10, and 50 μg/l for Zn, Cu, and Se determination. Correlation coefficients for calibration curves were always greater than 0.999. Matrix-matched calibration was used.

The analysis protocol assumed a 30-fold dilution of serum in the blank reagent. Blank reagent consisted of high purity water (>18 MΩ), TMAH (AlfaAesar), Triton X-100 (PerkinElmer), n-butanol (Merck), and EDTA (Sigma Aldrich).

The accuracy and precision of the measurements were tested using certified reference material (CRM), Clincheck Plasmonorm Serum Trace Elements Level 1 (Recipe, Germany).

Tab. 1. Clinical and biochemical characteristic of the study and control group

	Study group (n=35)	Control group (n=31)	*p*
Age [years]	8.83 ± 3.55	8.94 ± 3.64	0.8988
Glucose [mg/dl]	383.8 ± 145	84.9 ± 8.7	<0.05
HbA1c [%]	12.21± 2.46	5.01± 0.22	<0.05
Total Cholesterol [mg/dl]	164.2 ± 41.3	154.9 ± 23	0.7422
HDL [mg/dl]	46.6 ± 14.6	55 ± 13.2	0.0176
LDL [mg/dl]	101.4 ± 31	98.1 ± 22.1	0.6368
TG [mg/dl]	176.4 ± 237.1	74.9 ± 33.2	0.0038
Insulin [μIU/ml]	3.81 ± 3.36	-	-

HbA1c: glycated hemoglobin; HDL: high density lipoprotein; LDL: low density lipoprotein; TC: total cholesterol; TG: triacyloglycerol.

Islet autoantibody detection

The conventional islet autoantibodies were measured on serum samples as follows: islet-cell antibodies (ICA) with immunofluorescence, glutamic acid decarboxylase antibodies (GADA), tyrosine phosphatase-related islet antigen 2 antibodies (IA2A), and zinc transporter 8 antibodies (anti-ZnT8) by ELISA (RSR, USA), and insulin antibodies (IA) with radioimmunoassay (RSR, UK). The cut-off values for ICA, GADA, IA2A, IA, and anti-ZnT8 positivity were 10 Juvenile Diabetes Foundation (JDF) units, 10 U/ml, 20 U/ml, 7%/0.4 U/ml, and 15 U/ml, respectively.

According to the Islet Autoantibody Standardization Program (IASP), the sensitivity of the various antibodies in serum is as follows: ICA, 52–72.3%; GADA, 68–82%; IA2A, 66–70%; IA, 66%; and anti-ZnT8, 76%. The corresponding specificities were: ICA, 76–93.3%; GADA, 75–94.4%; IA2A, 47.6–70%; IA, 49.6%; and anti-ZnT8, 91.1%.

The ICA levels were determined in accordance with the International Workshop on the Standardization of ICA, an indirect immunofluorescence method, using a slice of human pancreatic tissue. The cut-off for ICA was 5–10 JDF units, depending on the substrate used. The sensitivity and specificity of the methods of antibody determination obtained by the Laboratory of Immunopathology and Genetics of KPOHiD UM in Lodz (LAB604) during the Islet AutoAntibody Standardization Program - IASP2015 for individual antibodies were: ICA, 72.0% and 94.4%; GADA, 82% and 98; IA2A, 70% and 95.6%; anti- ZnT8, 76% and 97.8%; and IA/IAA, 42% and 100%.

The ICA antibody was also analyzed using indirect immunofluorescence in monkey pancreas tissues (NOVA Lite ICA primate, pancreas slides, INOVA diagnostics, San Diego, CA, 92131, USA). In addition, the GADA, IA2A, IA, and anti-ZnT8 were analyzed using a commercial kit (RSR, UK). Overall, 160 tests were performed. In the 2008 diabetes antibody standardization program, the sensitivity of ICA was 98% and the specificity was 96%; the sensitivities of the GADA

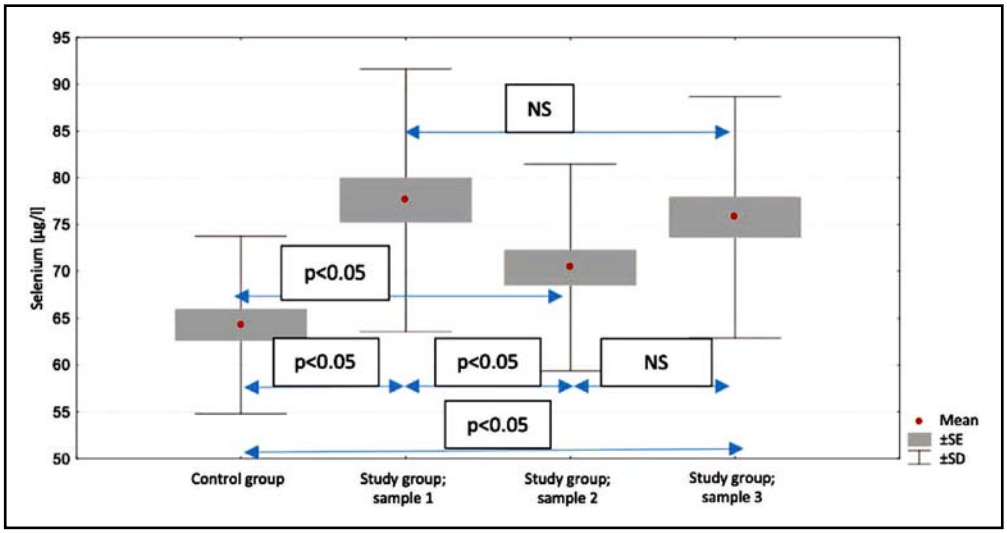

Fig. 2. Selenium concentration (μg/l) in the serum of children in the study (with DM1) and control (without DM) groups.
NS – non-significant; p – statistical significance; SD – standard deviation; SE – standard error.

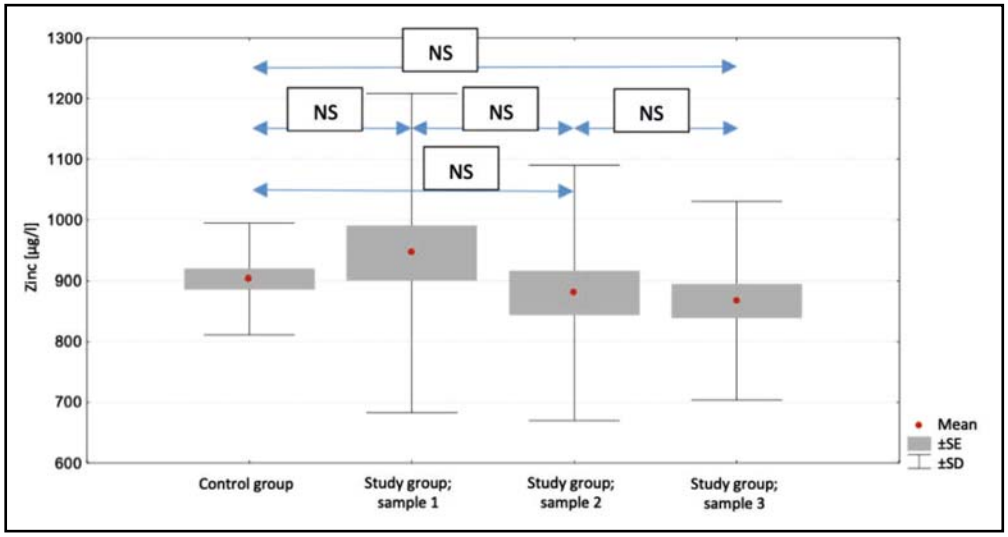

Fig. 3. Zinc concentration (µg/l) in the serum of children in the study (with DM1) and control (without DM1) groups.
NS – non-significant; p – statistical significance; SD – standard deviation; SE – standard error.

and IA2A were estimated at 88% and 74%, and the specificities were 98% and 98%, respectively.

Statistical analysis

Statistical analysis was performed with STATA 11. All continuous variables were tested for normality using the Kolmogorov–Smirnov test. These variables were described using means, standard deviations, medians, quartiles, and minimum and maximum values. The t-test or the Mann–Whitney U test was used to compare the differences between the two groups. Analysis of variance (ANOVA) or Kruskal-Wallis test was applied for many groups. Discontinuous variables are described by quantity and frequency of occurrence. The χ^2 Pearson test or Fisher's exact test were used to study the statistical relationships between discontinuous variables. Statistical relationships between discontinuous variables were assessed with Pearson's χ^2 test and described with a correlation coefficient (r) and probability (p). To estimate the risk of pathology depending on various factors, a logistic regression model was used. The results were described by giving the relative risk (OR) along with the 95% confidence intervals and the probability. The probability in this model was calculated using the χ^2 Pearson test or the two-sided exact Fisher's test. For all analyses, differences were considered to be statistically significant if *p*-values were less than 0.05.

RESULTS

Changes in glucose and glycated hemoglobin levels

The mean admission glucose levels were 383.8±145 mg/dl in the study group and 84.9±8.7 mg/dl in the control group (p=0.0000). The follow-up fasting glucose level in the study group (sample 3) was 146.3±55.0 mg/dl. Children with DM1 had significantly lower glycated hemoglobin levels at 6 months after diagnosis than at the time of diagnosis (6.65±0.56%).

Selenium

Selenium concentration on admission (sample 1) was significantly higher in the study group (77.61±14.03 µg/l) than in the control group (64.30±9.49 µg/l; p=0.0000). At the time of discharge (sample 2), the selenium concentration in the study group was lower than in sample 1 (p=0.0201) but higher than in the control group (p=0.0194). The selenium concentration in the study group upon 6 months control visit (sample 3) was lower than in sample 1, albeit not significantly (p=0.5754), and significantly higher than the control group (p=0.0001; Figure 2).

In both the study and control groups, significant correlations were observed between selenium levels and HbA1c (%) (r=0.39; p=0.0001) and concentrations of the following islet autoantibodies: ICA (r=0.41; p=0.0007), GADA (r=0.46; p=0.0001), IA2A (r=0.37; p=0.0025), and anti-ZnT8 (r=0.40; p=0.0009).

Zinc

The zinc concentration on admission in the study group (946±263.03 µg/l) did not differ significantly from that in the control group (903±92 µg/l; p=0.3910). The zinc concentrations were lower in samples 2 and 3 in the study group than in sample 1 and compared with the control group, although not significantly (Figure 3).

Copper

At the time of DM1 diagnosis, the copper concentration was significantly higher in the study group (1333±244 µg/l) than in the control group (1145±190 µg/l; p=0.0010). At the time of discharge (sample 2), the copper concentration in the study group

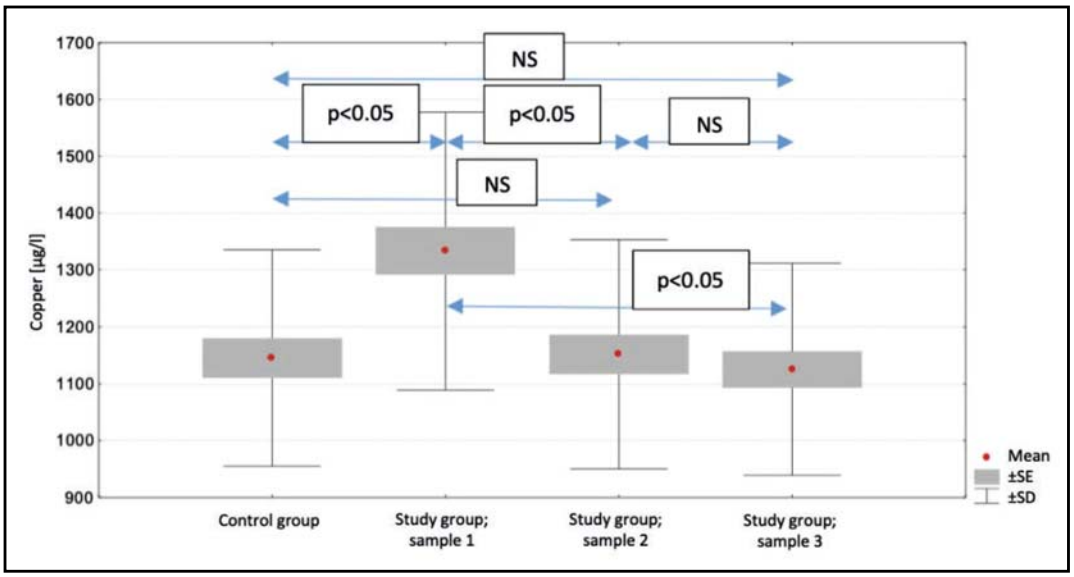

Fig. 4. Copper concentration (µg/l) in the serum of children in the study (with DM1) and control (without DM1) groups.
NS – non-significant; p – statistical significance; SD – standard deviation; SE – standard error.

was significantly higher than in sample 1 (p=0.0012). Upon 6 months control visit (sample 3), the copper level in the study group was significantly lower than in sample 1 (p=0.0002). The copper concentrations in samples 2 and 3 were lower in the study group than in the control group, albeit not significantly (Figure 4).

In both the study and control groups, significant correlations were found between copper levels and HbA1c (r=0.29; p=0.0029) and concentrations of the following islet autoantibodies: ICA (r=0.44; p=0.0002), GADA (r=0.30; p=0.0137), IA2A (r=0.33; p=0.0065), anti-ZnT8 (r=0.36; p=0.0032), and IA (r=0.31; p=0.0114).

Arsenic

Arsenic concentration on admission in the study group was 0.39±0.60 µg/l and did not differ significantly from that in the control group (0.25±0.35 µg/l; p=0.0634). The arsenic concentration in the study group was only slightly lower at discharge (sample 2) than in sample 1 (p=0.5145), but significantly higher than in the control group (p=0.0285). In sample 3, arsenic levels were higher than sample 1 (p=0.0103) and the control group (Figure 5).

Significant correlations were found between arsenic levels and HbA1c (r=0.27; p=0.0067) and ICA levels (r=0.30; p=0.0143) in the study and control groups.

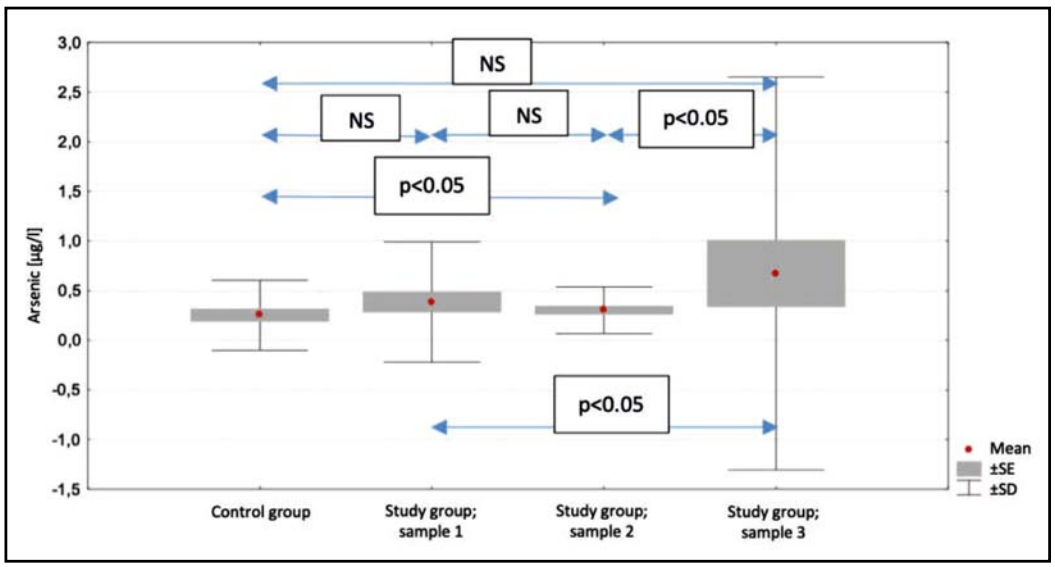

Fig. 5. Arsenic concentration (µg/l) in the serum of children in the study (with DM1) and control (without DM1) groups.
NS – non-significant; p – statistical significance; SD – standard deviation; SE – standard error.

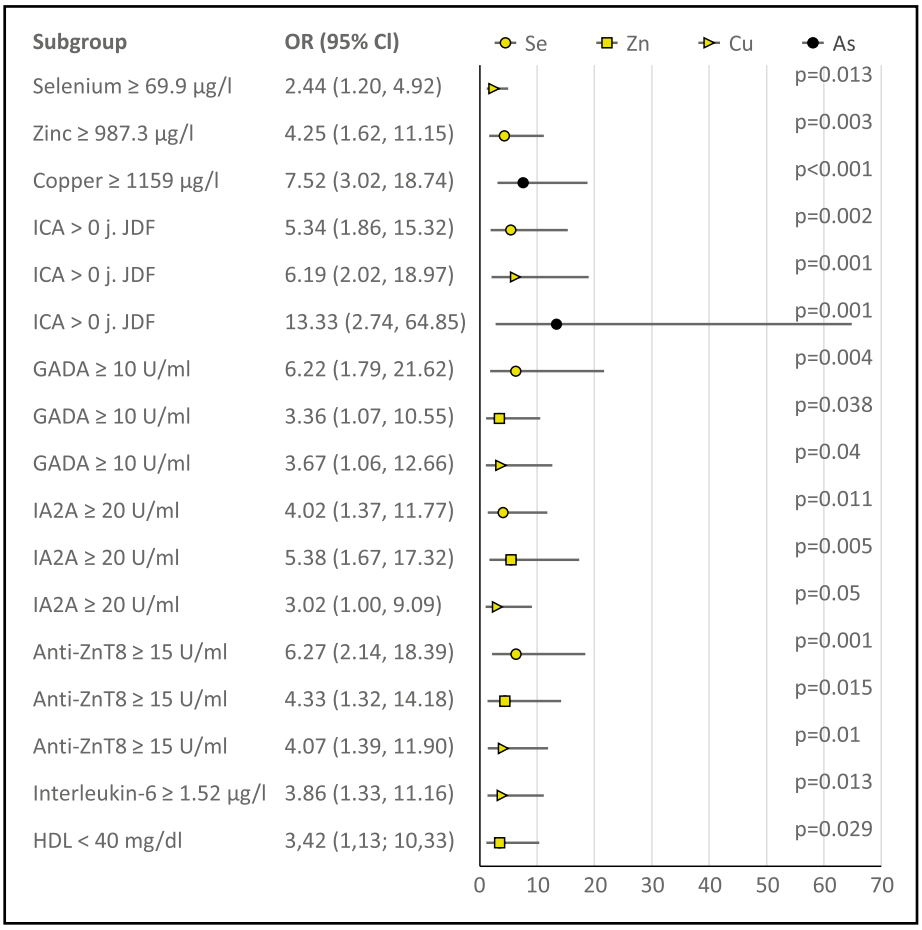

Fig. 6. Odds ratio (OR) for trace elements for different biochemical and immunological variables (95% confidence intervals and the probability).
Anti-ZnT8: zinc transporter 8 antibodies; GADA: anti-glutaminic acid decarboxylase antibodies; HDL: high density lipoprotein; IA2A: insulinoma- associated protein2 antibodies/ islet antigen2; ICA: islet cell antibody; p: statistical significance.

Odds ratio (OR) for trace elements for different biochemical and immunological variables is presented in figure 6.

DISCUSSION

The increasing prevalence of diabetes is hypothesized to be driven by environmental factors; however, the influence of trace elements on the development and pathogenesis of DM1 is unclear.

Children with DM1 (n=124; mean age, 12.8±4 years) were previously found to have significantly decreased selenium concentrations (Kruse-Jarres & Rükgauer 2000). Similar results were reported by Ruiz *et al.* (Ruíz *et al.* 1998), who examined 150 patients (age range, 11–60 years) with DM1. However, others found no significant change in selenium levels in 87 children with DM (mean age, 13±4 years; mean treatment duration, 3.5 years) compared with controls (Salmonowicz *et al.* 2014). Similar conclusions were reported in a study by Sobczak *et al.* (Sobczak *et al.* 2019) that examined 54 patients with DM1 (mean age, 26.3±6.8 years). In contrast, we found selenium concentrations were significantly elevated in all three samples in the study group than the control group. Moreover, selenium levels positively correlated with HbA1c and the concentrations of four islet autoantibodies: ICA, GADA, IA2A, anti-ZnT8 (fig.6). Conversely, Ruiz *et al.* (Ruíz *et al.* 1998) reported a negative correlation between selenium concentration and HbA1c. Furthermore, selenium supplementation did not reduce the risk of developing DM2 nor its complications in a previous study (Ogawa-Wong *et al.* 2016). Therefore, the risk of diabetes complications and selenium levels do not appear to have a direct linear relationship.

Zinc deficiency disrupts insulin homeostasis, resulting in decreased insulin secretion by β-cells (Fung *et al.* 2015). Indeed, zinc supplementation was found to improve insulin and glucose levels and reduce the risk of complications in patients with DM2 (Jayawardena *et al.* 2012; Islam *et al.* 2016; Ranasinghe *et al.* 2018). Moreover, zinc deficiency can induce a cytokine-mediated inflammatory response and contribute to the destruction of β-cells in the early stage of DM1 (Chausmer 1998). Indeed, children with DM1 were found to have had a significantly lower zinc concentration than healthy controls (Salmonowicz *et al.* 2014). Rohn *et al.* (Rohn *et al.* 1993) and Samadi *et al.* (Samadi *et al.* 2020) reported similar findings in 45 adults (mean age, 27.73±7.1 years) and 26 children with DM1. In contrast, Ruiz *et al.* (Ruíz *et al.* 1998), Kruse-Jarres *et al.* (Kruse-Jarres & Rükgauer 2000), and Sobczak *et al.* (Sobczak *et al.* 2019) found no significant differences in zinc concentrations among those with DM1

compared to controls. Likewise, we found no significant differences in zinc concentrations between the study and control groups. Moreover, although Samadi *et al.* (Samadi *et al.* 2020) showed that zinc concentration significantly correlated with HbA1c, we found no such correlation in our study. We confirmed zinc concentration was significantly positively correlated with three islet autoantibodies in children with DM1 (fig.6).

It has been proposed that zinc deficiency and an excess of copper can promote the development of diabetic complications (Ruíz *et al.* 1998; Navas-Acien *et al.* 2006; Salmonowicz *et al.* 2014). Zinc and copper homeostasis are closely related, as they compete with each other for intestinal absorption and both bind to albumin (Osredkar 2011). Indeed, decreased zinc levels and increased copper levels were observed in the serum of patients with poorly controlled DM1 (HbA1c >9%) (Lin *et al.* 2014). Additionally, studies by Salmonowicz *et al.* (Salmonowicz *et al.* 2014) and Kruse-Jarres *et al.* (Kruse-Jarres & Rükgauer 2000) found that diabetic patients had significantly higher copper concentrations compared with controls. In contrast, Rohn *et al.* (Rohn *et al.* 1993), Sobczak *et al.* (Sobczak *et al.* 2019), Ruiz *et al.* (Ruíz *et al.* 1998), and Samadi *et al.* (Samadi *et al.* 2020) demonstrated that copper levels were not significantly different between patients with diabetes and controls.

We found the copper concentration in children with DM1 at the time of diagnosis was significantly higher than in healthy controls. However, after treatment (i.e., at 2 weeks and 6 months), the copper concentrations were similar between the study group and the control group. Furthermore, copper levels have previously been shown to correlate with HbA1c (Kruse-Jarres & Rükgauer 2000; Lin *et al.* 2014), in men (Peruzzu *et al.* 2015) and women (Alimonti *et al.* 2005; Lin *et al.* 2014). Indeed, we confirmed copper concentration was significantly positively correlated with HbA1c and four islet autoantibodies in children with DM1 (fig.6).

Finally, arsenic concentrations may be implicated in diabetes. For example, significantly increased arsenic concentrations were observed in 309 people with DM2 in a study of 3,602 Koreans (aged ≥20 years) (Rhee *et al.* 2013). Similar results were reported by Grau-Perez *et al.* (Grau-Perez *et al.* 2018), who investigated the urinary arsenic concentrations in 1,451 Spanish people, 120 of whom had DM2. Coronado-Gonzales *et al.* (Coronado-González *et al.* 2007), Lai *et al.* (Lai *et al.* 1994), and Makris *et al.* (Makris *et al.* 2012), who carried out studies in Mexico, Taiwan, and Cyprus, respectively, also reached the aforementioned conclusion. Meanwhile, in a study of 42 people in Cambodia (mean age, 40.4±18.8 years), Huang *et al.* (Huang *et al.* 2014) found that 14 patients with DM2 had elevated urine arsenic concentration, although it was not statistically significant. Similar results were presented in a study conducted in Bangladesh (Rahman *et al.* 1998). Likewise, we found significantly elevated arsenic concentrations in children with DM1 after 2 weeks and 6 months of treatment compared with the control group. Exposure to low concentrations of arsenic was shown to induce oxidative stress responsible for disturbances in insulin secretion and β-cell function in DM2 (Makris *et al.* 2012); however, the effect of arsenic on the development of DM1 has not been studied. Although we found arsenic concentrations significantly positively correlated with HbA1c and ICA levels (fig.6), further research and analysis of arsenic levels in patients with DM1 is required.

Despite these important aspects of this study, the authors are aware of the limitations of their research, the small sample size in particular (35 participants). The statistical capacity of conclusions is influenced by the small size of the studied group. It should be noted that studies carried out in children are usually conducted on smaller clinical groups. Moreover, in the study there is a large gender disproportion of the surveyed children (as 80% of the group are boys and girls are only 20%), which may potentially affect the final results. It should be emphasized that the average age of the children examined by us is only 8 years. The study was prospective. It has been carried out in children with new onset type 1 diabetes, often in moderate to severe clinical conditions, whose parents are reluctant to consent to additional tests.

CONCLUSIONS

We found that there was no evidence of trace element deficiency among children with DM1 compared to healthy controls. The concentration of selenium was significantly increased among children with DM1 at all-time points investigated (i.e., at diagnosis, discharge, and upon 6 months control visit). Arsenic levels were only increased at the time of discharge and upon 6 months control visit. Meanwhile, copper levels were only significantly elevated at the time of diagnosis. We found no significant differences in zinc concentrations among children with DM1 compared to healthy controls or between time points, and no correlation was found between zinc concentration and ZnT8 antibodies. In summary, although trace elements in the environment (particularly selenium) may increase the incidence of DM1, this relationship requires further clarification.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, M.R.S., E.P. and M.W.; methodology, K.W., W.M. and J.L.; software, M.R.S.; validation, M.R.S., E.P. and H.R.; formal analysis, A.H.J, HC, and M.R.S.; investigation, M.R.S., H.R., D.K., H.C, A.H.J.

and E.P.; resources, M.R.S., D.K. and A.H.J.; data curation, M.R.S., K.W. and W.M.; writing—original draft preparation, M.R.S.; writing—review and editing, M.R.S., E.P., A.H.J and H.R.; visualization, M.R.S.; supervision, E.P.; project administration, M.W., J.L. and E.P.; funding acquisition, M.W. and H.C. All authors have read and agreed to the published version of the manuscript.

FUNDING

This research was founded by the statutory budget of the Department of Pediatrics, Endocrinology, Diabetology, Metabolic Disorders and Cardiology of Developmental Age of Pomeranian Medical University in Szczecin, Poland.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Pomeranian Medical University in Szczecin (no. KB-0012/34/17, 27.02.2017).

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study.

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