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Original Article

Serum Selenium Concentration in Patients with Autoimmune Thyroid Disease

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Abstract

Introduction: Selenium (Se) is one of the environmental factors with an essential role in the pathogenesis of autoimmune thyroid disease (ATD). Scarce data is available for the selenium status of the Bulgarian population especially for patients with thyroid disorders.

Aim: To compare the serum selenium (s-Se) concentrations in patients with ATD and healthy controls from Bulgarian population.

Materials and methods: The s-Se concentrations were measured in 105 patients newly diagnosed or untreated for the previous 6 months with ATD (mean age 44±13 years). The patients were divided into three groups: euthyroid autoimmune thyroiditis (AIT) (n=31), hypothyroid AIT (n=33), and hyperthyroid patients with AIT or Graves' disease (GD) (n=41). The results were compared to s-Se concentrations in 40 age- and sex-matched healthy controls. Determination of s-Se was carried out by inductively coupled plasma mass spectrometry (ICP-MS) after microwave-assisted acid mineralization of the serum samples.

Results: The s-Se concentrations in patients with hyperthyroidism were significantly lower than those in the control group (hyperthyroidism: 69±15.0 μg/L vs. controls: 84±13 μg/L, $p<0.001$). There was no significant difference in the s-Se concentrations between euthyroid and hypothyroid participants with AIT and healthy controls. The s-Se concentrations in our control individuals were within the range of 53-137 μg/L, reported in literature data on mean serum and plasma levels of European healthy adults.

Conclusions: The mean s-Se concentrations observed in all groups were below or close to the cutoff value of 80 μg/L, considered optimal for the activity of the Se-dependent antioxidant systems.

Keywords

autoimmune thyroid disease, Graves' disease, inductively coupled plasma mass spectrometry, serum selenium level, selenium deficiency

INTRODUCTION

Selenium (Se) is an essential trace element that is considered to have particular relevance to human health. It is involved in the synthesis of a large group of selenoproteins which contain the amino acid selenocysteine in their molecular structure. Many selenoproteins are vital for the body's antioxidant and immune defense, as well as for thyroid hormone metabolism.[1-3]

The thyroid gland is the organ with the highest content of Se mass fraction and retains it even in conditions of severe selenium deficiency.[3] In its cells, a number of selenoproteins such as glutathione peroxidases (GPx1 and GPx3) and thioredoxin reductases (TXNRD1, TXNRD2) are expressed. GPx1, GPx3, TXNRD1, and TXNRD2 catalyse the reduction of the excess hydrogen peroxide produced during thyroid hormone biosynthesis, thence providing local protection against oxidative stress and inflammation.[2-5] These processes affect the synthesis, activity, and metabolism of hormones, and play an important role in the pathogenesis of autoimmune thyroid disease (ATD).[6-8]

There is a lot of research highlighting the clinical relevance of selenium deficiency concerning the development of AIT, goiter, thyroid cancer, and GD.[5,9-14] The results of an extensive cross-sectional study (n=6152) conducted in two counties of China defined as adequate and low-selenium areas show a significantly higher prevalence of AIT in the low-selenium county.[11]

The effects of Se supplementation on autoimmune chronic thyroiditis and GD have been examined in a large number of observational studies and randomised controlled trials during the last two decades with inconsistent results. The general conclusion, as summarized in several systematic reviews, is that Se supplementation in doses of Se up to 100 μg/d reduces oxidative stress and the inflammatory process in the thyroid tissue.[15-19] This leads to an improvement of the thyroid function and morphology, as well as to a decrease in the antibody titre. However, Se supplementation appears to be beneficial in Se-deficient individuals and with potential health-related risks in those with an intake of excess concentrations.[20] Additional clinical data is necessary for confirmation of the clinical outcomes with a focus on the basal selenium status of the study populations. According to the current European Thyroid Association guidelines, selenium supplementation concerns only patients with Graves' orbitopathy, but a recommendation for patients with AIT is not yet included.[18,21]

In accordance with the above-mentioned facts, baseline selenium status is a decisive factor in assessing the need for selenium supplementation. However, it is extremely variable across the world, owing to a number of genetic and geographical factors, including the Se content in soil and the food intake.[20,22] As reported by a recent study of the Se content in Bulgarian soils and wheat grain, Bulgaria appears to be among the low-Se areas on the world Se atlas.[23] Furthermore, scarce data is available for the Se status of the Bulgarian population and even less for patients with thyroid disorders. The largest study in Bulgaria conducted about 30 years ago estimated s-Se reference ranges for adults with electrothermal atomic absorption spectrophotometry (ETAAS).[24] Previous studies determining s-Se in Bulgarian subjects were focused on certain population groups such as children pregnant and postpartum women.[13,25,26] An extensive population-based study in Bulgaria (n=2402) demonstrates the significant prevalence of hypothyroidism (6.3%) and hyperthyroidism (3.7%).[27] It has to be noted that in Bulgaria, effective prophylaxis of iodine deficiency has been implemented since the late fifties of the last century as reviewed by L. Ivanova.[28] This fact emphasises the importance of Se deficiency as one of the potential local environmental factors underlying thyroid dysfunction. The assessment of selenium status, though, requires a reliable biomarker. According to previous studies, the most commonly preferred laboratory parameters are serum/plasma Se concentration, determined with ETAAS or inductively coupled plasma mass spectrometry.[29]

AIM

In this prospective case-control study, we aimed to determine the s-Se concentrations in patients with ATD from a Bulgarian population.

MATERIALS AND METHODS

Patients and controls

The materials and procedures in the study were implemented in conformity with the international standards for ethics of health-related research involving humans and with the Helsinki declaration. The study was approved by the Regional Ethics Committee. All participants gave their written informed consent.

The study was conducted between 2017 and 2019 and included 145 participants aged 18-79 years from the Bulgarian population. A total of 105 patients with newly diagnosed or untreated ATD for the previous six months were divided into three groups: Group 1: euthyroid autoimmune thyroiditis (AIT) (n=31), Group 2: AIT with hypothyroidism (n=33), and Group 3: hyperthyroidism caused by AIT or Graves' disease (GD) (n=41). Exclusion criteria were severe and clinically significant chronic comorbidities. The categorisation into nosological diagnoses was made according to the internationally accepted diagnostic criteria based on history of disease, physical examination, ultrasound sonography, and laboratory tests. For comparison, we studied 40 randomly selected clinically healthy age- and sex-matched controls. None of the participants had an intake of Se-containing dietary supplements in the previous six months.

Blood sampling

Blood sampling was performed under fasting conditions following the standard procedure for collection of blood specimens. The blood samples were left to clot for 20 min at room temperature. After centrifugation at 3000 rpm for 10 min, serum subsamples were separated and stored at −70°C until analysis. For collection and storage of the samples, serum blood tubes (Kabe Labortechnik, Primavette V Serum, 2.6 mL) and cryotubes (Biosigma CL2ARBEPS CRYOGEN, 1.8 mL) were used. All labware used for blood sampling, storage, and analysis was previously tested for contamination with selenium, following a procedure described previously.[30]

Laboratory procedures

For the preparation of the calibration standard solutions multi-element standard (Etalon multi-element ICP, VWR Chemicals, Belgium), NaCl (CPAchem Ltd., Bulgaria), and Rhodium (Merck, Germany) were used. All solutions were prepared with ultra-pure water from ELGALabWater and nitric acid (suprapur) from Fisher Scientific UK Limited. The accuracy was checked with two levels of standard reference materials (Seronorm Trace Elements, SERO AS, Norway). Control and blank samples were prepared and measured simultaneously with every series of analysed samples. External Se pseudomatrix-matched standards were prepared in 15% v/v HNO_3 with a 130 mg/L Na content. Rhodium was used as internal standard.

Prior to measurement of s-Se, microwave-assisted acid digestion of the serum samples was performed by microwave digestion system with closed vessels (Multiwave GO, Anton Paar, Austria). Determination of Se was carried out by Thermo Scientific iCAP Qc ICP-MS (Thermo Scientific, Germany), equipped with a kit for online introduction of the internal standard. Thermo Scientific QTegra Software was used for calculations of the analytical results. The digestion procedure, the method for pseudomatrix-matched calibration, as well as the ICP-MS operating conditions, have been described in detail previously.[31]

Serum concentrations of hormonal parameters thyroid stimulating hormone (TSH, reference range: 0.34–5.6 mU/L), free triiodothyronine (FT3, reference range: 3.8–6.0 pmol/L), free thyroxine (FT4, reference range: 7.86–14.41 pmol/L), thyroglobulin antibodies (TgAb, reference range: 0–4 U/mL), and thyroid peroxidase antibodies (TPO-Ab, reference range: 0–9 U/mL) were measured by chemiluminescence immunoassay (CLIA) (Access 2, Beckman Coulter, USA).

Statistical analyses

IBM SPSS Statistics 19 and Microsoft Office Excel 2010 software products were used for statistical data analysis. The level of statistical significance was set at $p<0.05$. For the purpose of descriptive analysis, i) mean and standard deviation (SD) in the case of normally distributed values or ii) median and the range (10^{th}-90^{th} percentile), when deviation from normality was observed. The mean values of s-Se concentrations were compared by ANOVA and Dunnett's multiple comparison post hoc test. The Kruskal-Wallis test was applied for comparison of the s-Se concentrations between the TPO-Ab-negative group and the groups with a low and high level of TPO-Ab.

RESULTS

The demographics and clinical data of the studied groups are presented in **Table 1**.

The group of patients with hyperthyroidism showed significantly decreased s-Se concentrations compared to controls ($p<0.001$). This trend was not observed in patients diagnosed with hypothyroid and euthyroid AIT **(Fig. 1)** **(Table 2)**.

All patients were divided into three groups according to TPO-Ab titre in serum **(Fig. 2)**. Applying the Kruskal-Wal-

Table 1. Demographics and clinical data of the participants

Variables	Group 1 n=31	Group 2 n=33	Group 3 n=41	Controls (n=40)	*P*
Age (mean ± SD)	44±11	46±15	44±13	43±11	0.870*
Sex (male/female)	0/31	4/29	3/38	12/37	0.267**
TSH, mIU/L	2.5 (1.0–4.5)	12.1 (5.8–74.2)	0.003 (0.0–0.029)	2.2 (1.4–4.2)	<0.001*
FT3, pmol/L	4.8 (4.1–5.5)	4.8 (3.6–6.1)	13.7 (6.3–28.0)	5.1 (4.3–6.0)	<0.001*
FT4, pmol/L	10.7 (7.0–12.5)	9.4 (4.1–10.8)	37.0 (13.3–60.0)	10.0 (9.0–11.8)	<0.001*
TgAb, U/mL	8.5 (0.7–563)	4.9 (0.0–1859)	1.4 (0.0–340)	0.1 (0.0–1.5)	<0.001*
TPO-Ab, U/mL	351 (12.6–1011)	486 (7.9–1093)	151 (0.4–962.0)	1.2 (0.4–9.0)	<0.001*

Group 1: patients with euthyroid AIT; Group 2: patients with hypothyroid AIT; Group 3: patients with hyperthyroidism caused by AIT or GD; Variability of TSH, FT3, FT4, TgAb, and TPO-Ab is expressed as median (10th and 90th percentiles)

*The Kruskal-Wallis test was applied; ** The Fisher's exact test was applied.

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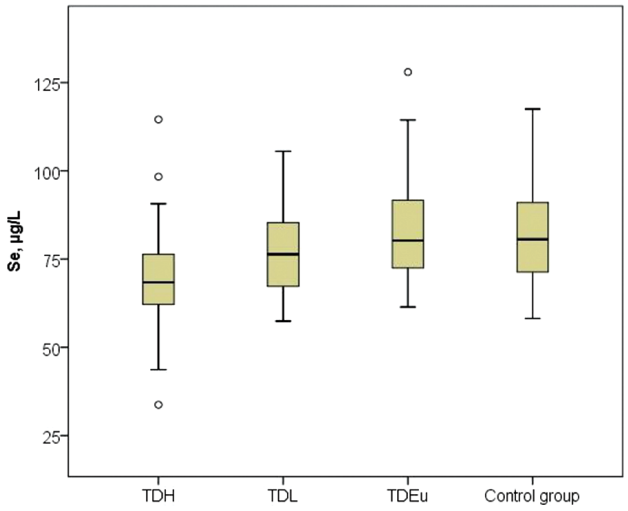

Figure 1. Distribution of the results of serum selenium concentrations in the compared groups (TDH: hyperthyroidism; TDL: hypothyroid AIT; TDEu: euthyroid AIT; CG: control group). ANOVA test was applied.

Table 2. Comparison of Se concentrations (mean ± SD) between participants of the control and patient groups

	N	Se µg/L	Patients groups vs. controls*
Euthyroid AIT	31	84±17	p=0.834
Hypothyroid AIT	33	77±13	p=0.334
Hyperthyroidism	41	69±15	p<0.001
Controls	40	82±13	–

*ANOVA with Dunnett's post hoc analysis was applied

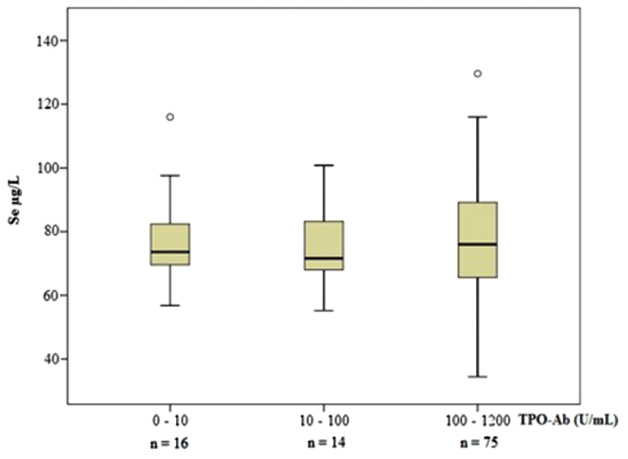

Figure 2. Comparison of s-Se between three groups of patients according to their TPO-Ab concentration in serum: TPO-Ab negative and TPO-Ab in low or high concentration. The Kruskal-Wallis test was applied (p=0.82)

lis test, it was found that there was no difference in s-Se values between the TPO-Ab-negative group and the groups with a low and high level of TPO-Ab (p=0.82).

DISCUSSION

Selenium is one of the environmental factors influencing thyroid function with a wide range of pleiotropic effects, ranging from antioxidant and anti-inflammatory effects to thyroid hormone synthesis.[2,3] There is evidence for the association of the Se deficiency and the prevalence of AIT and GD.[3,5,9-11,14] To the best of our knowledge, selenium status in population-based Bulgarian groups has been studied sparsely.[13,24-26] In the present study, we measured s-Se in Bulgarian group of patients with newly diagnosed ATD and compared the results with those for healthy controls.

Experimental evidence of the protective effects of Se against oxidative stress and cell damage in human thyrocytes and fibroblasts supports the claim that selenium deficiency is one of the manifest factors underlying the oxidant/antioxidant imbalance in ATD.[32] We found that s-Se concentrations were significantly lower in patients with hyperthyroid ATD (p<0.001) compared to the control group. There was no significant difference in s-Se concentrations between euthyroid and hypothyroid participants with AIT and healthy controls. The mean selenium levels in all groups are either below or very close to the cutoff of 80 µg/L considered to be required for maximal activity of GPx and selenoprotein P.[33] The results for the baseline s-Se concentrations in our patients with ATD (euthyroid AIT: 84 µg/L; hypothyroid AIT: 77 µg/L; hyperthyroid ATD: 69 µg/L) are consistent with those, reported in previous studies with patients residing in other European countries (AIT: 81-87 µg/L; GD: 74-75 µg/L).[9] However, these are lower than those previously observed in Italy and Denmark (AIT: 106-108 µg/L; GD: 90-91 µg/L).[9,14] Regarding the TPO-Ab, there were no statistically significant differences between the s-Se concentrations of our patients in the TPO-Ab-negative group and the groups with a low and high level of TPO-Ab, which is in agreement with previous studies.[9,14]

The mean s-Se concentrations demonstrated in our control individuals (82 µg/L) were within the range of 53–137 µg/L, reported in literature data on mean serum and plasma levels of European healthy adults.[34,35] However, these were higher than the median s-Se (57 µg/L) in the reference limits of s-Se concentration in the Bulgarian population (32–91 µg/L, n=294), reported by Tzatchev et al. in 1992.[24] Probable reasons for the different results in both studies could be the variable dietary habits of Bulgarians 25-30 years ago and the relatively lower number of participants in our study (n=40). Our findings have to be attributed to the fact that Bulgaria, as well as most European countries, is among the low-Se areas on the world Se atlas.[23]

It has to be noted that the observed suboptimal Se status in Bulgaria is under conditions of adequate iodine supple-

mentation owing to the implementation of a national program of iodine supplementation.[28] This fact further highlights the prominent role of Se deficiency in the prevalence of ATD in Bulgaria. Although the available evidence from controlled trials does not support routine Se supplementation as adjuvant therapy to standard treatment of patients with AIT or GD (except for mild GO)[18,21], the generally accepted opinion is that the correction of moderate to severe Se deficiency has a beneficial effect in the prevention and treatment of ATD.[15-19] In this regard, it is worth emphasizing that the baseline selenium status is a decisive factor in assessing the need for selenium supplementation. Therefore, the choice of an appropriate biomarker for its evaluation is of great importance. As serum/plasma concentration analysis is the most commonly used method for selenium determination[29,33], we suggest that s-Se concentrations in our study reliably reflects the selenium status in the studied sample.

CONCLUSIONS

In conclusion, this is the first study, so far, concerning the measurement of the selenium levels in newly diagnosed patients with ATD from the Bulgarian population which uses ICP-MS as an analytical method. Our findings demonstrate a significant decrease in s-Se concentrations in hyperthyroid subjects with ATD. They support the notion that selenium deficiency is one of the manifest factors underlying the oxidant/antioxidant imbalance in ATD, especially on the background of sufficient iodine supplementation. In this evaluation of s-Se of healthy Bulgarian subjects, we confirm previous data for low baseline Se concentrations in healthy Bulgarian population similar to most European countries. Our conclusions suggest that Se supplementation might have a beneficial effect in Bulgarian patients with ATD and could help provide national Se intake recommendations.

Author contributions

D.D.: conceptualization, selection of participants, collection of samples, methodology, investigation, writing - original draft preparation, statistical analysis; G.K.: methodology, investigation; M.M.: selection and diagnosis of patients; T.T.: conceptualization, writing - review and editing, supervision; M.O.: conceptualization, selection of participants, writing - review and editing; B.N.: selection and diagnosis of patients; K.S.: statistical analysis, writing - review and editing; V.K.: methodology, writing - review and editing.

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Competing Interests

The authors have declared that no competing interests exist.

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Концентрация селена в сыворотке крови у пациентов с аутоиммунным заболеванием щитовидной железы

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Резюме

Введение: Селен (Se) является одним из факторов окружающей среды, играющим важную роль в патогенезе аутоиммунного заболевания щитовидной железы (АЗЩЖ). Имеются скудные данные о статусе селена в болгарском населении, особенно у пациентов с заболеваниями щитовидной железы.

Цель: Сравнить концентрации селена (s-Se) в сыворотке крови у пациентов с АЗЩЖ и здоровых людей из болгарской популяции.

Материалы и методы: Концентрация s-Se измерялась у 105 пациентов с впервые диагностированной или нелеченной в течение предшествующих 6 месяцев АЗЩЖ (средний возраст 44±13 лет). Пациенты были разделены на три группы: эутиреоидный аутоиммунный тиреоидит (АИТ) (n=31), гипотиреоидный АИТ (n=33) и гипертиреоидные больные с АИТ или болезнью Грейвса (Базедовой болезнью (ББ) (n=41). Результаты сравнивали с концентрациями s-Se у 40 здоровых людей соответствующего возраста и пола. Определение s-Se проводили методом масс-спектрометрии с индуктивно-связанной плазмой (ICP-MS) после кислотной минерализации образцов сыворотки с помощью микроволнового излучения.

Результаты: Концентрация s-Se у пациентов с гипертиреозом была значительно ниже, чем в контрольной группе (гипертиреоз: 69±15.0 μg/L по сравнению с контрольной группой: 84±13 μg/L, $p<0.001$). Не было существенной разницы в концентрации s-Se между эутиреоидными и гипотиреоидными участниками с АИТ и здоровым участниками в контрольной группе. Концентрации s-Se у наших здоровых участников в контрольной группе находились в диапазоне 53-137 μg/L, что соответствует литературным данным о средних уровнях в сыворотке и плазме здоровых взрослых европейцев.

Заключение: Средние концентрации s-Se, наблюдаемые во всех группах, были ниже или близки к пороговому значению 80 μg/L, которое считается оптимальным для активности Se-зависимых антиоксидантных систем.

Ключевые слова

аутоиммунное заболевание щитовидной железы, болезнь Грейвса, масс-спектрометрия с индуктивно-связанной плазмой, уровень селена в сыворотке крови, дефицит селена