SELENIUM STATUS IN AUTOIMMUNE THYROIDITIS

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SELENIUM STATUS IN AUTOIMMUNE THYROIDITIS (Abstract): Selenium (Se) is an important element that exerts its effects through selenoproteins. The thyroid gland has the highest Se concentration and specific selenoprotein enzymes families are crucial in the thyroid hormone metabolism. There is little evidence on the link between Se and thyroid autoimmune disease, therefore future studies are required to elucidate the nature of this association. **Aim**: To evaluate the Se status in euthyroid subjects with autoimmune thyroiditis. **Material and methods**: From January 2014 to January 2015 we recruited 100 consecutive euthyroid subjects with autoimmune thyroiditis, living in the same region and with normal iodine intake. Serum concentrations of Se, thyroid antibodies (antithyroperoxidase – TPOAb – and antithyroglobulin – TgAb), thyroid-stimulating hormone (TSH), and thyroid ultrasound were performed in all patients. **Results**: Mean age of the study group was 48.87±12.83 years, range: 18-82 years. Since thyroid pathology is more frequent in the 5th – 6th decades of life we selected the age of 50 for the comparative analysis of the results (51% of patients were under 50). No statistical age-group differences in antibody levels were found: mean TPOAb = 420.95 IU/ml, p=0.840; mean TgAb = 327.98 IU/ml, p=0.977. TSH mean was 2.14 µIU/ml, with no significant age-group differences (p=0.176). Se levels ranged between 8.05 - 998.50 µg/ with a mean value of 294.96µg/L and no significant differences between age groups (p=0.158). Thyroid ultrasound showed inhomogeneity in 89%, nodules in 35% of patients, and a mean thyroid volume of 11.72ml, with no significant age-group differences (p=0.366). The low TSH levels were associated with low Se levels in 11.6% of cases, but the direct correlation was statistically insignificant (r = 0.116; R² = 0.0161; p = 0.371). Depending on TSH percentiles, mean Se levels showed no significant differences, however pointing out the highest mean value at the 25th percentile (F = 0.441, df = 61, p = 0.646). A negative correlation trend was found between Se and TPOAb (r=-0.2276) or TgAb (r=-0.2190) but lacking statistical significance (p=0.099). **Conclusion**: Our results showed a weak negative correlation between Se and antithyroid antibodies, suggesting that selenium supplementation may improve the course of thyroid autoimmunity. **Keywords**: SELENIUM, AUTOIMMUNE THYROIDITIS.
Selenium (Se) was first isolated in 1817 by Jacob Berzelius (1), and its name derives from Selene – goddess of the moon. Se is a metalloid present in the environment, water, soil and air, generally in very low concentrations (< 1µg/g) (1) and is an essential element for human health.

Se has a concentration-dependent effect in the body, being an antioxidant at nanomolar or micromolar levels while in higher concentrations it has a prooxidant effect.

The normal plasma Se concentrations of range between 60 and 120µg/l or 0.8 ± 0.36µmol/l being related to its intake through diet and the daily dose should not exceed 400 µg/day, dose at which toxic effects were observed (2).

Se deficiency can affect human health in different ways. The severe endemic deficiency is associated with Keshan disease (congestive cardiomyopathy) and Kaschin-Beck disease (chronic, endemic osteochondropathy), and the mild one with various limited expression of Se-dependent enzymes. Causes of deficiency are dietary low intake and poor intestinal absorption. Selenosis is the most common disease as a result of over limit intake of Se from food (3).

The majority of Se in tissues is found in proteins (seleno-proteins, selenotrisulfides and other acide-labile Se compounds) (4). In humans, the thyroid gland has the highest Se concentration as a constitutive part of Dio 1 and 2 that convert thyroxine (T4) to triiodothyronine (T3); Gpx 1 and 4, which protect thyreocytes against H2O2 released during thyroglobulin iodination; Gpx3, regulating H2O2 concentration in the lumen of thyroid follicle; selenoprotein P and Txnrd 1, having an antioxidant effect (5).

In normal nutritional conditions, the liver is the primary source of plasma T3, due to liver deiodination of T4 by Dio 1 (which actually contributes to the release of T3 by the thyroid). In Se deficiency, the primary source of T3 is the thyroid by increasing thyroid activity of Dio1 which allows maintaining normal concentrations of plasma T3 despite reduced activity of hepatic Dio (6).

Selenium deficiency leads to a decrease in GPx activity that results in the accumulation of H2O2 in the thyroid and thus enhancing apoptosis. Since H2O2 is essential in the synthesis of T3 and T4, its accumulation could be the activator of increased thyroid production of T3 and T4. Probably Dio 1 is required to maintain an optimum T4/T3 balance in these conditions (6).

Selenium was shown to have an important role in lymphocytic chronic thyroiditis. Most authors consider that Se affects the autoimmune system by controlling the production of ROS (Reactive Oxygen Species) and Se supplementation determines a stimulating activity on glutathione peroxidases and thioreductases. Another path in which Se is involved in autoimmune thyroiditis may be by inhibiting the expression of HLA-DR molecules (7, 8).

Currently, there are no ways to stop the autoimmune process in chronic thyroiditis; levothyroxine treatment only addresses to the consequences of autoimmune aggression (9).

Given current knowledge, selenium therapy to reduce aggression and to prevent autoimmune thyroid dysfunction, remains the only therapeutic alternative, whose effectiveness needs to be supported by more evidence.

**MATERIAL AND METHODS**

We conducted a one-year prospective study (January 2014 - January 2015) in the
Selenium status in autoimmune thyroiditis

Department of Endocrinology of the “St.Spiridon” Hospital Iasi. One hundred consecutive subjects with euthyroid autoimmune thyroiditis and normal iodine intake were selected. Serum concentrations of TPOAb, TgAb, thyroid-stimulating hormone (TSH), Se and thyroid ultrasound were performed in all patients. In a second phase, Se was randomly administrated to half of these patients and re-assessed them after 3 months.

**Inclusion criteria:** female patients aged 18 to 2 years with normal thyroid function (TSH =0.4–4 µIU/ml) and autoimmune thyroiditis (TPOAb>35 UI/ml or/and TgAb>40 UI/ml) able to sign informed consent. All patients included in our study signed the informed consent. **Exclusion criteria:** major co-morbidities making it difficult to participate in the study, thyroid treatment (LT4 or anti-thyroid drugs) previous or at the time of enrollment; pregnancy or breast-feeding; malabsorption or consumptive syndromes, other autoimmune diseases, and intake of selenium supplementation above 70 µg/day; inability to read or understand Romanian; or lack of informed consent.

**Variables studied:** age, sex, titer of TPOAb and TGAb, TSH, volume (V) and echostructure (E) at thyroid ultrasound, and plasma Se levels.

TSH and thyroid Abs were measured by chemiluminescence at the hospital laboratory.

Se measurements were performed by atomic absorption spectrometry using the GF with platform HR-CS-AAS contra 600 AnalytikJena (10). The method we used involves several steps: fresh blood samples were preserved by freezing at -25°C; they were defrosted and homogenized before metal digestion; metal digestion for Se analysis also had several steps. A mixture of 1 ml total blood with 3 ml of nitric acid 65% and 1 ml hydrogen peroxide 30% was allowed 15 minutes to react. To realize the digestion process we kept the samples at 145°C for 5 minutes, at 190°C for 10 minutes and finally at 100°C for 10 minutes. After the digestion process the samples were transferred into 25 ml decontaminated Duran volumetric flasks. The flasks containing the samples were filled with ultrapure water up to a volume of 25 ml, shaked few times and then the content was transferred in 15 ml bottles for the final metal analysis.

The collected data were processed and interpreted by using the SPSS 18.0 software and statistical and mathematical tests.

**RESULTS**

Mean age of the study group was 48.87±12.83 years, range: 18-82 years. The study group was divided into two groups according to their age, with age 50 as the demarcation point, justified by the fact that thyroid pathology is more frequent after the 5th–6th decade of life. 51% patients were aged under 50 years. No statistical age-group differences in antibody levels were found: mean TPOAb = 420.95 IU/ml, p = 0.840; mean TgAb = 327.98 IU/ml, p = 0.977. For TSH the mean value was 2.14 µIU/ml with no significant age-group differences (p=0.176). Se levels ranged between 8.05-998.50 µg/L with a mean value of 294.96 µg/L (p=0.158). Thyroid ultrasound showed inhomogeneity in 89% of patients, nodules in 35%, and a mean thyroid volume of 11.72 ml (p=0.366) (tab. I).

Depending on TSH percentiles, mean Se levels showed no significant differences, however pointing out the highest mean value at the 25th percentile (F = 0.441, df = 61, p = 0.646). A negative correlation trend was found between Se and...
TPOAb (r=-0.2276) (Fig.1) or TgAb (r=-0.2190), but lacking statistical significance (p=0.099) (fig. 2).

The low TSH levels were associated with low Se levels in 11.6% of cases, but the direct correlation was statistically insignificant (r = 0.116; R^2 = 0.0161; p = 0.371) (fig. 1).

The same result (negative Se-ATPO Ab correlation) was obtained when we divided our study group into 3 subgroups depending on Se levels (tab. II).

### TABLE I

<table>
<thead>
<tr>
<th></th>
<th>Se (µg/L)</th>
<th>TSH (µIU/ml)</th>
<th>TPOAb (IU/ml)</th>
<th>TgAb (IU/ml)</th>
<th>VT (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50y</td>
<td>256.76 246.70 (8.05-998.50)</td>
<td>1.860 0.769 (0.569-3.67)</td>
<td>328.97 348.75 (10-1000)</td>
<td>337.83 595.29 (10-2372)</td>
<td>11.00 7.98 (0.83-47.7)</td>
</tr>
<tr>
<td>&gt;50y</td>
<td>344.48 229.58 (25.08-771.25)</td>
<td>1.777 0.949 (0.4-3.93)</td>
<td>326.95 359.96 (10-1000)</td>
<td>478.23 569.93 (10-1946)</td>
<td>12.47 8.20 (3.91-44.41)</td>
</tr>
<tr>
<td>Total</td>
<td>294.96 241.48 (8.05-998,50)</td>
<td>1.830 0.864 (0.4-3.93)</td>
<td>327.98 352.49 (10-1000)</td>
<td>440.08 609.23 (10-2372)</td>
<td>11.72 8.08 (0.83-47.07)</td>
</tr>
</tbody>
</table>

**Fig. 1.** Correlation between Se and TSH

**Fig. 2.** Correlation between Se and TPOAb

### TABLE II

<table>
<thead>
<tr>
<th></th>
<th>TSH</th>
<th>ATPO</th>
<th>Se vs TSH</th>
<th>Se vs ATPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>t test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mVSN</td>
<td>0.839941</td>
<td>0.885068</td>
<td>0.19813</td>
<td>-0.43639</td>
</tr>
<tr>
<td>mvsM</td>
<td>0.86379</td>
<td>0.276061</td>
<td>-0.00761</td>
<td>-0.12693</td>
</tr>
<tr>
<td>nvSM</td>
<td>0.430623</td>
<td>0.420775</td>
<td>0.130626</td>
<td>-0.14692</td>
</tr>
</tbody>
</table>

m : Se <60 µg/L, n: Se = 60-120 µg/L , M: Se >120 µg/L

**DISCUSSION**

Se is an essential micronutrient in many aspects of human health, which plays a major part in optimal endocrine response, immunomodulation and inflammatory process. The most frequent disease involving the thyroid gland is chronic autoimmune thyroiditis, an inflammatory process which progressively destroys the gland. Several studies have examined the potential benefits of Se in this disease to assess if its supplementation may be effective in in-
Selenium status in autoimmune thyroiditis

In the present study we measured Se levels in the blood of euthyroid patients with autoimmune thyroiditis. This is the second study which provides information on Se status in Romanian patients with autoimmune thyroiditis. The fist data on Se level in the Romanian patients were provided by a multicenter study published in 2014 (12) which recruited patients from four European countries (Greece, Romania, Austria, Italy) (tab. III).

<table>
<thead>
<tr>
<th>TABLE III</th>
<th>Selenium status in different European countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author</td>
<td>Country</td>
</tr>
<tr>
<td>Krassas, Pontikides, Tziamalos et al.</td>
<td>Italy (2014)</td>
</tr>
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<td></td>
<td>Greece (2014)</td>
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<td></td>
<td>Austria (2014)</td>
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<td></td>
<td>Romania (Timisoara 2014)</td>
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<tr>
<td>Present study, The effect of Se supplementation on antioxidant status, hormonal, autoimmune and ultrasonographic profile in euthyroid patients with chronic autoimmune thyroiditis</td>
<td>Romania (Iasi 2015)</td>
</tr>
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</table>

When comparing the results of these two studies we found that the Se levels reported in 2015 were higher than those from the multicenter study. This difference can be explained by the fact that we used total blood which contains more Se than plasma. In our study Se was determined by atomic absorption spectrometry (see methods). The method we used is an original one but there are various methods for Se determination: nuclear activation analysis (NAA), electrothermal atomic absorption spectrometry (ET AAS), hydride generation atomic absorption spectrometry (HG AAS), fluorimetry, X-ray fluorescence analysis (XFA), and gas-liquid chromatography (GCL) (Bem EM). Chromogenic reagents used for Sedetermination by various spectrophotometric methods are: dithiozone, chromotropic acid, J-acid, Variflavin Blue (12).

Usually, these methods are used to determine Se in plasma, serum, whole blood and other tissues. Se concentration in the serum and plasma have similar values (plasma contains 81% of the Se of whole
blood and 94% of serum) and correlates with Se ingestion. Se in whole blood varies less and correlates better with GPx(3). The obtained Se levels, even if the same tissue is used, can vary depending on method. Another variable is the number of patients (the 2015 Iasi study had a greater number of patients) and the heterogeneity of the study group. The iodine intake is also very important. Anamnestically, our patients had a normal iodine intake. Previous studies revealed that Moldavia is an iodine deficient region, except Iasi, where the iodine intake is normal (13).

Levels of Se also vary by geographic region (type of soil) which influences the dietary Se intake. The dietary Se intake is in the range 7-4990 mg/day, with different mean values for each geographical area (40μg/day in Europe, 93μg/day in women and 134μg/day in men in the USA). Also, the dietary Se concentrations are dependent on the soil Se content, form and distribution of Se in foods (Se from plant source foods is a more bioavailable form than animal source foods) (13).

China, India, Middle–East and some European countries have a very low content of Se in soil resulting in Se deficiency in the local population (3). Soil of the driest regions concentrate high quantities of Se and alkaline soils release more Se than the acid ones (3).

Selenium content of foodstuffs varies in a broad range between countries, depending mainly on selenium content in soils and its availability (bioavailability) for plants. The bioavailability for plants depends mainly on the chemical forms of selenium in the soil, but is also influenced by some physicochemical parameters of the soil. The absorption of Se⁶⁺ is generally higher than that of Se⁴⁺. The selenium content in animal products depends on its content in the diet consumed by the animals (14).

In most countries, legumes, cereals and derivatives are the main source of selenium intake, due to their predominance in the diet. In few cases, meat, poultry and seafood are the main contributing food categories to selenium intake (14).

Agricultural activities can influence Se levels in foodstuffs. In some areas of the world Se has been added to fertilizers in order to increase Se levels in cultivated plants and indirectly improve selenium status in humans (3).

As to our study it is difficult to integrate the results of Se determinations because we donot have the normal Se levels for our population and we donot know the content of Se in our soil. So we compared our results with those reported by other European countries (Table III). The different results are accounted for by the differences in dietary intake and soil content.

It is difficult to obtain unbiased estimates of dietary trace-element intake, no matter of the epidemiological method used. Due to high variability in Se content of different foodstuffs and interindividual diet variations, the procedure for estimating Se dietary intake is questionable (14). The recommended adult daily Se intake varies considerably between authors, countries and organizations. Schrauzer and White (cited by Dharmasena, 2014) estimated a daily Se intake per person in the range 90-168 μg. In Asia, Africa and many European countries the daily Se intake is below the recommended dose. Se deficiency has negative consequences mostly in seniors, pregnant and lactating women, growing and developing children (3). In order to provide a safe total Se intake the reference dose (RfD) from all nutritional sources for a 70 kg adult has
been set at 350µg/day, corresponding to 5 µg Se/kg body weight/day (11).

We also found that Se status correlated positively with age, which is in line with the findings from most similar studies (11).

Regarding TPO Abs, the available data show that in 11 Se supplementation trials in patients with autoimmune thyroiditis, 7 have shown benefit and positive effects on serum TPO-Ab concentrations. Another fact is that high Se levels in the blood are associated with low TPO levels (15).

Our study revealed a negative correlation between Se and TPO – Ab but lacking statistical significance (p=0.099). Our study group was divided into 3 groups according to Se level (and compared them with the normal Se levels in Europe - plasma Se =60g/L -120g/L). We found no significant differences between TSH and ATPO values depending on Se level. Instead, for low Se levels we obtained a negative correlation with ATPO-Ab, which suggests the possible benefits of selenium treatment in autoimmune thyroiditis (tab. II).

New studies are required to evaluate the evolution of antithyroid antibodies during Se supplementation and the development of reliable methods for direct determination of Se level in the body and especially the amount necessary to ensure homeostasis and prevention of thyroid specific autoimmune pathology. Also, little is known about the effect of mild selenium deficiency in the context of normal iodine intake (situation specific for our country).

**CONCLUSION**

In our study the low Se levels were associated with high TSH levels, suggesting a possible involvement of selenium in thyroid function.

Our results showed a weak negative correlation between selenium and antithyroid antibodies, so selenium supplementation may improve the evolution of thyroid autoimmunity.

Meaningful clinical outcomes should be demonstrated before Se supplementation can be routinely suggested in patients with thyroid autoimmunity.

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**REFERENCES**

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CARDIAC REHABILITATION AFTER SURGICAL CORRECTION OF CONGENITAL HEART DISEASE

Comprehensive cardiac rehabilitation is an important element of the comprehensive management of grown-up congenital heart disease patients after surgical correction of congenital heart disease but access to this treatment is still limited. To assess the effect of a comprehensive cardiac rehabilitation program on physical capacity, exercise tolerance, quality of life, and severity of depressive symptoms in grown-up congenital heart disease patients long-term after surgical correction of congenital heart disease. Implementation of a comprehensive cardiac rehabilitation program improves physical capacity, exercise tolerance, and quality of life and reduces depressive symptoms in patients late after surgical correction of congenital heart disease. Introduction of such programs seems reasonable as a supplement to the holistic care for grown-up congenital heart disease patients (Gierat-Haponiuk K, Haponiuk I, Szalewska D, Chojnicki M, Jaworski R, Niedoszytko P, Leszczyńska K, Bakula S. Effect of complex cardiac rehabilitation on physical activity and quality of life during long-term follow-up after surgical correction of congenital heart disease. Kardiol Pol. 2015;73(4):267-73).

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