Selenium- Essential Antioxidant Element
The example of autoimune thyroiditis

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To evaluate the effect of Selenium (Se) supplementation on: thyroid stimulating hormone (TSH), antiperoxidase antibodies (TPOAb) and glutathione peroxidise 1 (GPx1) in euthyroid subjects with autoimmune thyroiditis. 100 euthyroid women with autoimmune thyroiditis, from the same region, were randomized to receive daily 100 μg selenomethionine (n=50) or placebo (n=50) for 3 months. Serum concentrations of Se, TPOAb and TSH were performed in all patients at baseline and after 3 months. GPx1 activity was measured only in the interventional group before and after Se supplementation. At 3 months TSH presented a significant increase both in treated (2.49 vs. 2.09 UI/mL; p=0.001) and untreated groups (2.38 vs. 1.91 UI/mL; p=0.008). TPOAb decreased by 15.2% in patients treated with Se (p=0.002) and were not modified in untreated patients. At the end of the study Se and TPOAb were in direct insignificant correlation (r=+0.267, p=0.105). GPx1 did not show significant changes after Se supplementation. After 3 months of Se supplementation results showed a mild decrease of TPOAb and a weak negative correlation of these antibodies with Se levels. This suggests that Se treatment may improve the course of thyroid autoimmunity.

Keywords: Selenium, glutathione peroxidase, autoimmune thyroiditis, oxidative stress.

Se is an essential element for human health being involved in redox processes, intracellular signaling, autoimmunity, apoptosis and cell proliferation [1, 2]. This is possible due to the activity of at least 30 selenoproteins variants encoded by 25 human genes [3]. Several selenoproteins are located in the thyroid gland: thioredoxin reductases (TRs), three deiodinase isoforms (D1, D2 and D3) and glutathione peroxidase (GPXs). TR1 has an antioxidant effect. D1 and 2 convert thyroxine (T4) in tironine (T3). GPX1 and 4 protect thyreocites from damage caused by oxygen free radicals [4]. Selenium exhibits anti-inflammatory and antioxidative actions and was shown to have an important role in lymphocytic chronic thyroiditis [5, 6]. Se affects the autoimmune system by controlling the production of ROS (Reactive Oxygen Species) [7] and also may indirectly inhibit TNF activation and cytokine release [5]. The inhibition of the expression of HLA-DR molecules is possible to be another explanation of the protective effect of Se in thyroid autoimmune process. All this data suggests the possible relationship between the Se deficiency and autoimmune thyroiditis and the Se supplementation has been considered in this regard [8]. Nowadays, there are no ways to stop the autoimmune process in chronic thyroiditis and the treatment with levothyroxine is necessary only in the stage of hypothyroidism [9]. The results of clinical studies regarding the effect of Se in thyroid autoimmunity are discordant but it is true that the dose used varies widely as well as the duration of treatment [10, 11]. Since the normal plasma Se concentrations varies between 75 and 150 μg/L or 0.8 ± 0.36 μmol/L, related to the dietary intake (under 400 μg/day due to toxic effects), [12] it is very difficult to establish the right amount of Se or the duration of therapeutical intervention necessary to induce clinical effects. Anyway, most clinical trials showed that 100-200 ìg/day of selenomethionine or sodium selenite for 3 to 12 months was related to the decrease of TPOAb titers [13]. Taking into account that in several regions of Romania the soil content in Se is very low [14] and given current knowledge that selenium therapy might reduce aggression and prevent autoimmune thyroid dysfunction (remaining the only actual therapeutic alternative), its effectiveness needs to be supported by more evidences [15]. Based on these data, we wanted to evaluate the efficacy of Se supplementation in patients with autoimmune thyroiditis and normal thyroid function in terms of serum levels of TPOAb and glutathione peroxidase activity.

Experimental part

Patient and methods

This randomized-controlled trial was conducted between January 2014-January 2016 in the Department of Endocrinology of the Hospital St. Spiridon, University of Medicine and Pharmacy *Grigore T. Popa*, Iasi. Patients were eligible if they had euthyroid autoimmune thyroiditis. The diagnosis was assessed by the presence of detectable TPOAb levels and normal TSH. One hundred women with euthyroid autoimmune thyroiditis and normal iodine intake were randomized into 2 groups (Group I, n=50, treated with selenomethionine 100ìg/day, Group II, n=50, control). Serum concentrations of TPOAb, TSH and Se were performed in all patients at baseline and after 3 months. Serum levels of GPx1 were determined only for the treated patients at baseline and after 3 months. Inclusion criteria: adult female patients, with normal thyroid function (TSH = 0.4-4 μIU/mL) and autoimmune thyroiditis (TPOAb> 35 UI/mL), able to sign informed consent. Variables studied: age, titers of TPOAb, TSH, GPx1 and plasma Se levels. The study protocol was approved by the Ethics Committee of our institution (University of Medicine and Pharmacy, Iasi) and the written informed consent was obtained from all participants. Serum levels of TSH (reference values 0.4-4

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µUI/ml), TPOAb (0-35 UI/mL) were measured by chemiluminescence (Human TSH, TPOAb CLIA Kit). Se measurements were performed by atomic absorption spectrometry using the GF with platform HR-CS-AAS contra 600 Analytic Jena. 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 1ml total blood with 3 mL of nitric acid 65% and 1ml hydrogen peroxide 30% was allowed 15 minutes to react. To realize the digestion process we kept the samples for 5 min at 145°C, 10 min at 190° and 10 min at 100°C. 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, shacked few times and then the content was transferred in 15 ml bottles for the final metal analysis. Se concentrations were expressed in µg/L. GPx1 activity was determined using a commercially available kit (RANSEL control based on Paglia and Valentine method –

ELISA Kit for Glutathione Peroxidase 1 from Cloud-clone Corp®). The values of GPx1 were expressed in mU/dL.

Statistical analysis was performed using SPSS software version 18 (SPSS Inc., Chicago, IL, USA). The results are presented as mean ± standard deviation (SD).

Results and discussions

Of the 123 recruited patients, 100 women fulfilled the inclusion criteria and were enrolled in the study. 50 women (mean age 46.24±12.50) were randomized to receive selenomethionine and 50 women (mean age 50.50 ±13.48) were randomized to receive placebo. Table 1 summarizes the results: TSH, TPOAb, GPx1 and Se concentrations at baseline and after 3 months. There were no significant differences in mean serum levels of TSH at baseline between the two groups. After 3 months, TSH presented a significant increase in group I (2.09 *vs* 2.49 UI/mL; p=0.001) and in group II (1.91 *vs* 2.38 UI/mL; p=0.008), but in respect with normal ranges. Mean serum TPOAb levels at baseline was significantly higher in treated group vs placebo (362.99 *vs* 284.79 UI/mL; p=0.045).This distribution was a consequence of the randomization. Individual values of TPOAb in patients treated with Se after 3 months, were in indirect correlation with initial values, with an important decrease of TPOAb in 20% of cases (r= -0.196; R^2= 0.038; p=0.125) (fig. 1). The dynamics of average values in the TPOAb showed a decrease by 15.2%, statistically significant in patients treated with Se (p=0.002), which did not occur in untreated patients (p=0.850). Mean serum levels of GPx1 in group I at baseline and after 3 months were not significant different (0.64±0.37 vs 0.64±0.38 mU/dL, p=0.979). At baseline mean Se level was similar in the two groups and after 3 months significantly increased in group I (560.14 vs 270.50; p=0.001) but also in placebo group (316.13 vs 236.49; p=0.014) even if to a lesser extent. Considering normal values of Se between 75-150µg/L we have divided both

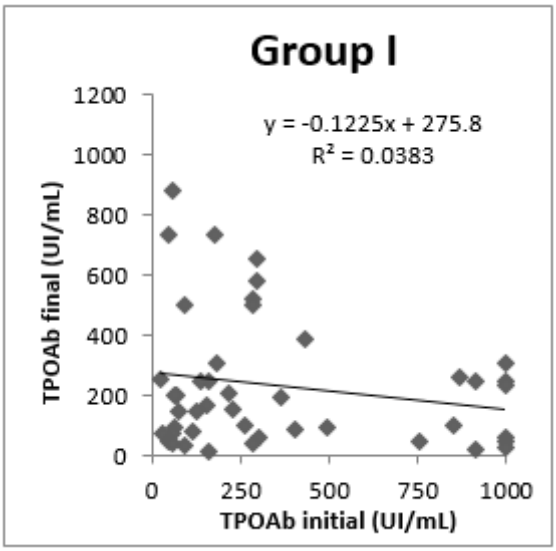

Fig. 1. Evolution of individual values of TPOAb in Se-treated group between baseline and end of study.

Table 1

BASELINE AND 3 MONTS BIOLOGICAL PROFILE (TSH, ATPOAB, SE AND GPX1) OF THE PATIENTS WITH CHRONIC AUTOIMMUNE THYROIDITIS RANDOMISED TO SELENOMETHIONINE OR PLACEBO

	GROUP I (n=50)	GROUP II (n=50)	p value
Selenomethionine supplementation (µg/day)	100	0	-
TSH (µUI/ml)			
- at baseline	2.08±1.00	1.91±1.06	0.392
- at 3 months	2.49±1.27	2.38±1.30	0.677
TPOAb (UI/ml)			
- at baseline	362.99±348.24	284.79±235.06	**0.045**
- at 3 months	307.87±306.1	289.96±287.78	0.781
Se (µg/L)			
- at baseline	257.69±240.51	236.49±211.63	0.652
- at 3 months	560.14±363.09	316.13±160.27	**0.001**
GPx1 (mU/dl)			
- at baseline	0.64±0.37	-	-
- at 3 months	0.64±0.38	-	-

Table 2
VARIATIONS IN TPOAB ACCORDING TO THE SERUM CONCENTRATION IN SE

Marker	Group 1				Group 2			
	Se<75	75-150	Se>150	p	Se<75	75-150	Se>150	P
Baseline								
TPOAb	439±88	428±116	264±63	0.023	396±157	292±78	242±61	0.577
3 Months								
TPOAb	*	164±68	201±42	0.049	306±180	317±90	271±57	0.501

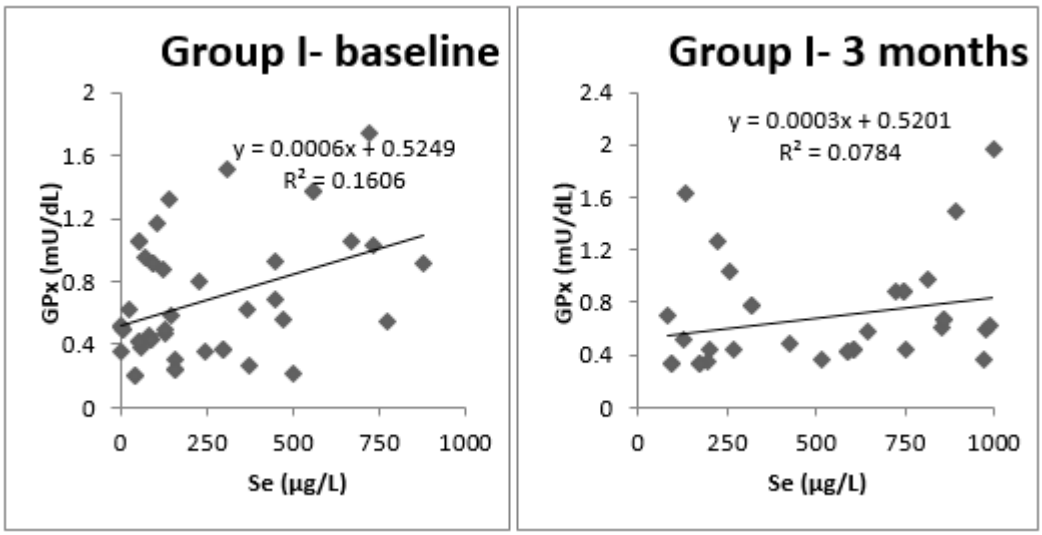

Fig. 2 Correlation between GPx1 and Se in group I (direct, significant at baseline and non-significant after 3 months of treatment)

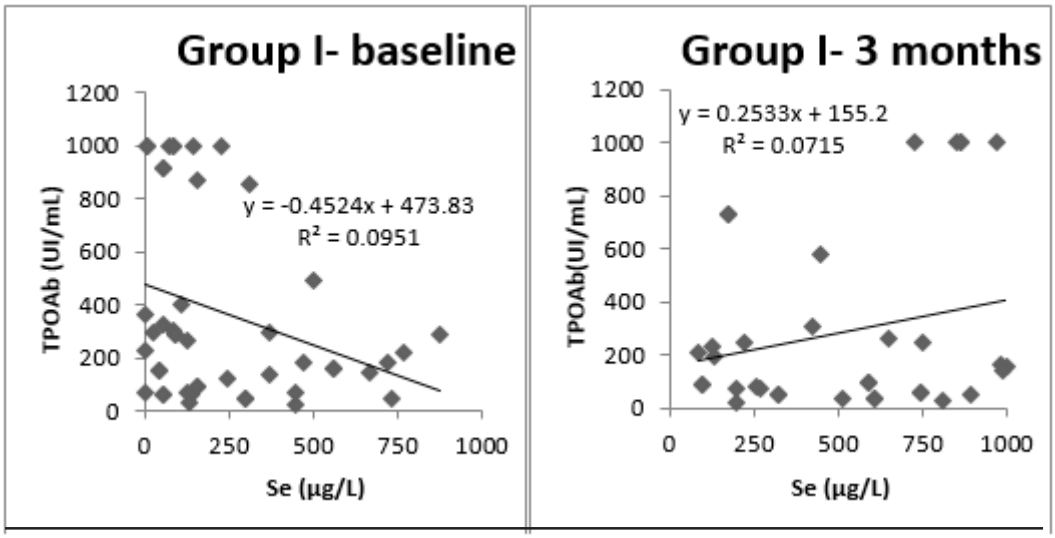

Fig. 3. Correlation between TPOAb and Se in group I (direct, significant at baseline and non-significant after 3 months of treatment)

groups in three categories: < 75, 75-150 and >150 µg/L. In group I, patients with Se levels < 75 µg/L, at baseline, presented the highest levels of TPOAb and those with Se > 150µg/L, on the contrary, the lowest concentrations of antibodies (p=0.023). The same tendency was present in the placebo group. After 3 months, in group I, the concentration of TPAb decreased with the increase of Se levels, this fact was not noticed group II. Also, the concentrations of GPx1 showed the same pattern. All these data are summarized in table 2. At baseline, in the group I the individual values of Se and GPx were in significant direct correlation, moderate in intensity (r= +0.401; R^2= 0.1606; p=0.006) (fig. 2). After 3 months of treatment, the individual values of Se and TSH were in a weak indirect correlation, statistically insignificant (r= -0.232; R^2= 0.0537; p=0.161) in the group I and independent in the group II (r= +0.024; R^2= 0.006; p=0.866). In the treated group, the individual values of Se and TPOAb at baseline showed an indirect correlation with a moderate but significant intensity (r= -0.308; R^2= 0.0006; p=0.037) which decreased in intensity and was statistically insignificant at the end of the study (r= +0.267; R^2= 0.0715; p=0.105) (fig. 3).

In the placebo group Se and TPOAb were independent values at baseline (r= -0.086; R^2= 0. 0074; p=0.553) and at 3 months (r= +0.014; R^2= 0.0002; p=0.924) (fig. 3).

Our results showed a mild decrease of TPOAb and a weak negative correlation between Se and antithyroid antibodies suggesting that Se supplementation may improve the course of thyroid autoimmunity. There is no consensus regarding the dose of Selenium useful in autoimmune thyroiditis – the dose that would be effective by reducing the intensity of the immune response without being toxic. The majority of previous studies demonstrate the effectiveness of a 200 μg Se per day [16]. Some immune functions were enhanced by a 50 μg Se, but the activity of the glutathione peroxidase, which is involved in other immune processes such as thyroiditis was not. The optimization of the immune function seems to appear at 100μg/day. Taking into account the potential adverse effects of the excessive supplementation of selenium such as insulin resistance [17], we chose the 100 μg over 200 μg Se/day. The medication was well tolerated; none of the patients reported side effects or intolerance phenomena.

The effect of Selenium supplementation on TPOAb

After 3 months of Se treatment, a significant decrease, by 15.2%, was observed in the TPOAb titer, which was not noticed in the untreated group. If the antibody titer is very high at the initiation of treatment, Se supplementation was proved more efficient since there was a significant decrease in the ATPO level [16, 18]. The same effect was observed in our study. As a consequence the indirect correlation between Se and TPOAb present at baseline was no longer valid at the end probably due to an amelioration of the autoimmune process. An important issue is the dose and duration of treatment. Three months of 100 μg/day Se may not be enough to normalize the level of TPOAb. Esposito et al [19] showed that 166 μg/day of Selenomethionine for six months has a limited impact on TPOAb. It seems that 200μg/day for 6-9 months have a greater impact on autoimmune process [16].

The effect of Selenium supplementation on TSH

There are different opinion regarding the impact on Se treatment on TSH values: Pirola et al [4] report the restoration of euthyroidism in patients with sub-clinical hypothyroidism after 4 months of 83 Selenomethionine μg/day. The decrease of TSH was also mentionated by Winther et al [3], in a population of 361 subjects with autoimmune thyroiditis under Se for a period of 5 years. In our study, even if low values of TSH had a tendency to correlate with selenium level, this correlation was not statistically significant concordant with most of the literature studies [13]. However, we noticed a growing tendency of TSH, which was statistically significant, both in the treated and the untreated women, while the TSH remained within the normal limits. Increased TSH in treated patients has been found in other studies and was attributed to T3 decreament due to the decrease in the activity of peripheral deiodinases [20].

The effect of Selenium supplementation on GPx1

Previous studies have shown that the administration of sodium selenite induced a dose proportional increase in the GPx1 activity [21, 22]. A minimum intake of 65μg/day was needed to optimize and 95ìg/day to maximize the GPx1 activity [23]. It is considered that the daily selenium intake must provide 2/3 of the maximum GPx activity [24]. A comparaison between studies is difficult because GPx1 normal values vary depending on the detection method as well as on the population investigated. Our results showed that mean serum levels of GPx1 at baseline and after 3 months were not significantly different in patients with euthyroid autoimmune thyroiditis after Se supplementation. A tendency towards parallelism was noticed at baseline between GPx and TSH values (lower TSH values corresponded to lower GPx values), but this correlation was not significant. At the end of the study, the tendency of the correlation reversed with lower TSH values and higher GPx values, which indicated an improvement of the function; however, this tendency was not significant. The GPx1 and TPOAb individual values were statistically independent both at the beginning and at the end of the study. This lack of correlation may be explained by the dose which we used (100μg/day), since the dose necessary to fill in the deficit storage in GPx1 is greater than the supplementation dose [16].

Conclusions

Our study demonstrated the existence of an indirect correlation between ATPO and the value of selenium. Selenium supplementation induces a decrease in antibody titer, more significant if the initial values were high. Selenium supplementation did not result in functional changes (TSH). GPx was directly and significantly correlated with the value of selenium, both at baseline and after 3 months, suggesting an improvement in oxidative stress.

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