



THE IMPORTANCE OF SERUM SELENIUM CONCENTRATION MEASUREMENTS IN PATIENTS WITH MILD THYROID-ASSOCIATED ORBITOPATHY RECEIVING ADJUVANT SELENIUM SUPPLEMENTATION – A PILOT STUDY

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The essential trace element selenium (Se) exerts complex effects on the endocrine and immune systems, partly due to its antioxidant capacity. The importance of an optimal Se intake has been established in autoimmune thyroiditis. A recently published study reported beneficial results in Se supplemented patients with mild Graves' orbitopathy. Eight female patients with mild Graves' orbitopathy and three female patients with mild orbitopathy based on Hashimoto thyroiditis participated in the study. The status of the orbita was registered at the beginning of the study and one-six months thereafter both according to the scores recommended by the American Thyroid Association (ATA) and clinical activity scores (CAS). The patients were treated according to their thyroid hormone status, their antibody results and their CAS. Moreover, they received adjuvant 100 or 200 µg Se as sodium-selenite. Serum Se concentrations were measured before Se supplementation was implemented and 1-6 months after the beginning of treatment. ATA scores improved in 10 patients and worsened slightly in one female patient. CAS improved in all study participants. Mean Se concentrations were $75.08 \pm 11.55 \mu\text{g L}^{-1}$ before supplementation was started and $91.35 \pm 17.37 \mu\text{g L}^{-1}$ one-eight months later ($p < 0.05$). At the beginning of the study, the majority of patients exhibited elevated antibody levels. After conventional treatment and adjuvant Se supplementation, antibody levels decreased or reached the normal range (except in the case of one patient).

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INTRODUCTION

Graves' orbitopathy is an autoimmune inflammatory disorder affecting the orbit around the eye, in particular the connective tissue and the external eye muscles. It is part of a systemic process caused by autoantibodies. It accompanies Graves' disease in about 10-30% of cases and is about 2-3 times more frequent in women than in men. The disease is characterized by upper eyelid retraction, swelling, redness, and bulging eyes. About 10% of patients do not have Graves' disease, but do have autoantibodies. Cigarette smoking raises the incidence significantly.^{1,2}

Graves' orbitopathy can present in mild, moderately severe and active forms. The activity of the disease can be assessed by using the criteria of the American Thyroid Association (ATA) and/or the Clinical Activity Score (CAS). CAS may range from 1 in a mild case to maximum 10 in the most active form³. There are effective treatments available for the moderately severe and active forms, including therapy with glucocorticoids and orbital irradiation, or both^{4,5}. Unfortunately, these therapies have many side effects and are avoided in mild forms of the disease which may often resolve spontaneously. However, many patients with mild Graves' orbitopathy have a substantial decrease in their quality of life.⁶ Therefore, it would be advantageous to

treat patients with mild Graves' orbitopathy with an affordable, well-tolerated and widely available agent.

Selenium, an essential trace element with antioxidant and immunomodulatory properties, has been recently shown by the European Group on Graves' Orbitopathy to significantly improve quality of life, reduce ocular involvement and slow progression of the disease in patients with mild Graves' orbitopathy in a 6-month study.⁷ However, serum selenium concentrations were not measured in those 54 patients that were given sodium selenite. Our study, in contrary, focused on both the course of the disease in patients with mild Graves' orbitopathy and on the measurement of their serum selenium levels at the beginning of and during treatment.

METHODS

Eleven patients with thyroid-associated orbitopathy (eight female patients with mild Graves' orbitopathy and three female patients with mild orbitopathy based on autoimmune thyroiditis) participated in the study. All study participants were recruited at the Polyclinic of the Hospitaller Brothers of St. John of God in Buda, Budapest, Hungary.

After the Ethics Committee had approved the study, informed consent was obtained from all study participants in writing before collection of blood samples. Approximately 10 ml of blood was collected from each individual after 12 hours of fasting. Blood was taken in metal-free glass tubes free of anticoagulant, using the standard venipuncture technique. Blood samples were allowed to clot and then samples were immediately centrifuged for 10 min at 2400

rpm. Serum was separated into metal-free plastic tubes and stored frozen at $-80\text{ }^{\circ}\text{C}$ until analysis, on average for 2-3 months.

The status of the orbit was registered at the beginning of the study and one-six months thereafter both according to the scores recommended by ATA and CAS. The patients were treated conventionally according to their thyroid hormone status, their antibody results and their CAS. Moreover, they received adjuvant 100 or 200 μg Se as sodium-selenite. Serum Se concentrations were measured before Se supplementation was implemented and 1-6 months after the beginning of treatment. Clinical characteristics of all study participants including their smoking status are shown in Table 1.

Table 1. Clinical characteristics of study participants

	Study participants (n=11)
Age mean \pm SD (years)	44.6 \pm 15.2
Diagnosis: Graves' disease	8
Diagnosis: Hashimoto thyroiditis	3
Smokers	6
Length of disease (years)	0-10

Laboratory parameters were determined on a Hitachi 917 instrument. Thyroid-stimulating hormone (TSH) was determined by a method based on chemoluminescence.

Free thyroxin (fT4), free triiodothyronine (fT₃), thyrotropin receptor antibodies, anti-thyroglobulin antibodies (anti-Tg), and anti-thyroid peroxidase antibodies (anti-TPO) were measured using ECLIA.

Sample preparation for selenium determination was carried out in duplicate using the nitric acid - perchloric acid - sulphuric acid digestion procedure recommended by Hershey et al⁸. After digestion, selenium (VI) was reduced to selenium (IV) with hydrochloric acid. Serum selenium concentration was determined by atomic absorption spectrometry following hydride generation on a Solaar M5 AA Spectrometer, Thermo Elemental equipment. The analytical method was verified by analysis of a human reference serum.

Statistical analysis was performed using the Microsoft Excel program. Results were considered significant if $p < 0.05$.

RESULTS AND DISCUSSION

All participating subjects exhibited mild thyroid-associated orbitopathy. According to their ATA scores, the cornea and vision were not affected in any of the patients. Clinical activity scores ranged from 2 in the mildest case to 5 in the most severe case.

Mean Se concentrations were $75.08 \pm 11.55 \mu\text{g/l}$ before supplementation was started and $91.35 \pm 17.37 \mu\text{g/l}$ one-eight months later ($p < 0.05$) which indicates that patients adhered to therapy. Moreover, it can be concluded that out of the many commercially available selenium compounds a drug was chosen whose absorption was effective.

ATA and Clinical activity scores improved in all 11 patients although some patients were treated only for a relatively short time interval as their symptoms improved rapidly and they did not need constant surveillance.

At the beginning of the study 4 patients had hyperthyroidism according to their TSH-levels, one patient had an elevated TSH-value, while TSH-levels were normal in 6 patients. TSH levels normalized in 3 patients during treatment.

Initially, all patients exhibited elevated thyrotropin receptor antibodies, nine study participants had elevated anti-thyroglobulin antibody (anti-Tg) and seven patients had high anti-thyroid peroxidase antibody levels (anti-TPO). After receiving conventional treatment and adjuvant Se supplementation for 4 months on average, thyrotropin receptor antibody levels decreased in all patients, anti-thyroglobulin antibody and anti-thyroid peroxidase antibody levels decreased in 10 and 11 patients, respectively.

Selenium methionine has been applied with convincing results in patients with autoimmune thyroiditis for the past 10 years^{9,10}. The exact mechanism of the benefit achieved with selenium is not entirely clarified. The antioxidant properties of selenium as part of the selenoenzyme glutathione peroxidase¹¹ should exert a profound effect. However, recently selenium was also reported to have a dose-dependent inhibitory effect on the expression of HLA-DR molecules of thyrocytes induced by interferon- γ which indicates that the beneficial effect of selenium on autoimmune mechanism might be due to very complex mechanisms¹².

Similarly to autoimmune thyroiditis, the beneficial results reported in selenium-supplemented patients with thyroid-associated orbitopathy are difficult to explain. It should be noted, however, that most treated patients to date came from areas in which selenium levels are known to be marginally decreased in the general population⁷. This is also the case in Hungarian healthy blood donors who have been shown to be selenium-deficient¹³ and Hungarian pregnant women who were reported to have very low serum selenium concentrations¹⁴. However, the frequent measurement of serum selenium concentrations in Se-supplemented subjects is of utmost importance and should always be performed in order to prevent Se overdose.

In summary, serum selenium concentrations increased in study participants while ATA scores and Clinical Activity Scores improved. Patients tolerated selenium supplementation and their compliance was adequate. There were no registered side effects during the study.

This is the first Hungarian study administering adjuvant Se supplementation of patients with mild thyroid-associated orbitopathy. Based on our results, patients currently enrolled in the study will continue Se supplementation for another 6 months.

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