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Myo-inositol plus selenium supplementation restores euthyroid state in Hashimoto's patients with subclinical hypothyroidism

M. NORDIO, S. BASCIANI

Department of Experimental Medicine, University of Rome "Sapienza", Rome, Italy

Abstract. – OBJECTIVE: Clinical evidence suggests that oral supplementation with myo-inositol (MI) and selenium (Se) is useful in the treatment of autoimmune thyroiditis. The purpose of this study was to highlight the positive response of Hashimoto's patients with subclinical hypothyroidism (SH) treated with MI and Se (MI-Se) in restoring a normal thyroid function.

PATIENTS AND METHODS: A total of 168 patients with Hashimoto's thyroiditis (HT) having Thyroid Stimulating Hormone (TSH) levels between 3 and 6 μIU/ml were randomized into 2 groups: one receiving MI-Se and the other one Se alone.

RESULTS: TSH, anti-thyroid peroxidase (TPOAb) and anti-thyroglobulin (TgAb) levels were significantly decreased in patients treated with combined MI-Se after six months of treatment. Also, a significant free serum T4 increase was observed in MI-Se group, along with an amelioration of patients' quality of life.

CONCLUSIONS: The administration of MI-Se is significantly effective in decreasing TSH, TPOAb and TgAb levels, as well as in enhancing thyroid hormones and personal wellbeing. Such treatment restored euthyroidism in patients diagnosed with autoimmune thyroiditis.

Key Words:
Myo-inositol, Selenium, Autoimmune thyroiditis, TSH, TPOAb, TgAb, Hashimoto's thyroiditis, Subclinical hypothyroidism.

Introduction

Chronic autoimmune thyroiditis (AIT) is one of the most common autoimmune diseases in iodine-sufficient areas, affecting more than 10% of females and 2% of males in the overall population. Although this illness may often occur in children and teenagers, it mainly affects 30-50 years old people[1]. Elevated Thyroid Stimulating Hormone (TSH) and autoantibodies such as anti-thyroglobulin (TgAb) and anti-thyroid peroxidase (TPOAb) are typical features of AIT. In such pathologies, among which Hashimoto's thyroiditis (HT) is the most representative, thyroid gland gets progressively underactive, due to cell and antibody-mediated autoimmune processes[2]. A mild thyroid failure is called subclinical hypothyroidism (SH), and usually is balanced by a slight TSH level increase, approximately between 3-6 μIU/ml. According to the American Thyroid Association's (ATA's) guidelines, the normal range for TSH values, with an upper limit of 4.12 μIU/ml is largely based on National Health and Nutrition Examination Survey (NHANES) III data, but it has not been universally accepted. In fact, some have proposed that the upper normal TSH values should be either 2.5 or 3.0[3]. TSH, a glycoprotein secreted by the hypophysis, regulates the hypothalamic-pituitary-thyroid axis by harmonizing the release of thyroid hormones[4]. It plays several key physiological roles, from the promotion of thyroid epithelial differentiation and growth[5], iodide uptake and transport[6], to protection of thyroid cells from apoptosis[7]. Particularly, on thyroid follicular cells, TSH binds to the TSH receptor, stimulating cell growth and differentiation, in addition to thyroid hormone synthesis. This binding with TSH receptors activates adenylyl cyclase, leading to an increase of intracellular cyclic AMP and protein Kinase A phosphorylation, and also to an activation of cytosolic and nuclear target proteins. At high doses, TSH stimulates the phospholipase C-dependent inositol phosphate Ca^{2+}/diacylglycerol pathway, resulting in a boost of hydrogen peroxide (H_2O_2) generation[8]. Besides, it has been shown that impairment of TSH signal transduction leads not only to thyroid disorders such as hypothyroidism and hyperthyroidism, but also to the proliferation and differentiation of human thyroid carcinoma cells[9].

Corresponding Author: Maurizio Nordio, MD; e-mail: maurizionordio1@gmail.com

Currently, a specific treatment for AIT has not been found yet, however, various studies have demonstrated that L-thyroxine (L-T4)[10] and selenium (Se)[11] may moderately lower antibodies serum concentrations in HT patients. Particularly, in a randomized, placebo-controlled prospective study the administration of Se plus LT4 was beneficial to AIT patients, reducing serum TPOAb and TSH levels[12], although L-T4 efficacy is quite controversial in patients with already low TSH levels[13]. Mazokopakis et al[14] have shown that Se significantly decreased the serum TPOAb concentrations after a 12 months treatment period. Another study demonstrated that 12 months of 80 μg sodium selenite administration to AIT patients reduced TPOAb and TgAb concentrations, whereas TSH and free thyroxine (fT_4) levels were unvaried[15]. Likewise, 200 μg sodium selenite per day were able to decrease serum thyroid TPOAb autoantibodies levels after 3 months of treatment in autoimmune thyroid patients[16]. Furthermore, in the same clinical trial, the Se effect was evaluated in improving the patients' wellbeing, as subjects with AIT normally have impaired health-related quality of life[16].

Se is an essential trace element for humans; selenium-dependent enzymes exert antioxidant and anti-inflammatory properties[17]. It has a relevant impact on immune function[17]; *de facto*, it has been shown to reduce the inflammatory status in patients with AIT[16]. Se-dependent enzymes, such as Glutathione Peroxidase (GPx), substantially have the ability to diminish hydrogen peroxides, lipid, and phospholipid hydroperoxides; in this way, they limit the propagation of free radicals and reactive oxygen species, resulting in a reduction of inflammatory prostaglandins (PGs) and leukotrienes[18,19].

In a previous study[20] from our laboratory, the co-treatment with L-selenomethionine plus myo-inositol (MI) demonstrated a beneficial effect on SH, restoring a euthyroid state and reducing both TPOAb and TSH levels, bringing them closer to physiological levels.

MI is a carbocyclic polyol precursor of phosphoinositide synthesis; it is involved in cell signaling[21] and, precisely, as a second messenger regulating the activities of several hormones such as TSH, follicle-stimulating hormone (FSH) and insulin[22]. Indeed, in TSH signal cascade, inositol regulates hydrogen peroxide-mediated iodination[23].

Based on this clinical evidence and our previous preliminary data, we decided to further investigate the efficacy of a combined administration of MI and Se in AIT to evaluate whether this treatment could restore euthyroid state and improve personal well being in HT patients.

Patients and Methods

A total of 168 outpatients (mean age 40.85 ± 8.5 years) with HT were enrolled in this prospective randomized controlled trial (RCT) from March 2015 to February 2016. Each participant filled in the informed consent. This study was approved by the ethics committee of the Italian Society of Phytotherapy and Supplements in Obstetrics and Gynecology (SIFIOG). Inclusion criteria were: age range 22-62, TSH levels between 3-6 μIU/ml, elevated serum TPOAb and/or TgAb, normal fT_4 level (0.6-1.8 ng/dl) and free triiodothyronine (fT_3) (2.0-3.5 pg/ml). Exclusion criteria were: debilitating illnesses (e.g. depression, psychosis, severe hepatic or renal failure); malabsorption disorders (atrophic gastritis, celiac disease); adjuvant treatment with vitamins or trace elements. The primary outcome was the reduction of serum TSH levels. The secondary outcomes were the restoration of TPOAb, TgAb, fT_3 and fT_4 hormone concentrations as well as the quality of life evaluation.

Patients were randomized 1:1 into 2 groups: 84 subjects (control group: A) including 10 men and 74 women, and 84 subjects (experimental group: B), with 9 men and 75 women. The calculation of the sample size determined that a minimum of 52 patients were required in each group to have an acceptable statistical power. At baseline, both groups had similar values for age, and TSH, TPOAb, TgAb, fT_3 and fT_4 hormone levels. Controls (group A) were given 16.6 mg L-selenomethionine (corresponding to 83 μg Se) in tablets, and patients in group B were given tablets containing 600 mg MI plus 16.6 mg L-selenomethionine (= 83 μg Se) (Tiroxil®, LO.LI. Pharma Srl, Rome, Italy). Patients were asked to take the supplement with water about 2 hours before or after the meal, for six months. Subjects were treated daily by oral route and asked to bring back the empty boxes, used for the treatment, at the following scheduled medical visits. Also, a questionnaire to evaluate the level of subjective symptomatology (SS), due to the thyroid inflammation process, was filled in by each patient at the beginning and the end of the study.

Laboratory and Technical Investigations

From the 171 patients selected, 168 agreed to enter the study. Three of them were excluded when they got pregnant during the recruiting period.

Blood samples were drawn from each patient and serum TSH, fT_3, fT_4, TPOAb, and TgAb levels were measured at baseline and the end of the 6-month period. The concentrations of fT_3, fT_4 and TSH were measured by an enzyme immunometric assay (Byk-Sangtec Dietzenbach, Germany). Plasma total TPOAb and TgAb concentrations were measured by a commercial enzyme luminescence assay (Byk-Sangtec, Dietzenbach, Germany). The specificity for AIT in these assays is greater than 90% when antibody concentrations are above 350 IU/ml.

To provide additional information concerning thyroid tissue texture, high-resolution ultrasound scan of the thyroidal area was carried out using Doppler sonography[24]. Particular attention was paid to evaluate the so-called Systolic Peak Velocity (SPV) of the lower thyroid artery because its blood flux generally is altered during the inflammatory process. A single experienced operator, who was unaware of the diagnosis, performed all the scans.

Atomic absorption spectrometry was carried out to determine plasma selenium. Plasma MI was determined using Gas Chromatography-Mass Spectrometry (GC-MS) analysis after extraction with organic solvents and derivatization. Injection (1.0 μl) was performed in splitless mode at 270 °C and a capillary column Agilent 122-5532 DB-5ms (0.25 mm × 30 m × 0.25 μm) was used. Total run time was 15 minutes: oven at 70 °C from 0 to 1 min; 20 °C /min to 150 °C; 10 °C/min to 240 °C; 4 min at 320 °C in post-run. The flow rate was fixed to 1.2 ml/min and results were analyzed by an MS 5973 Network Series detector in SIM mode.

At enrolment and after 6 months the SS was evaluated using a questionnaire[25], which includes 7 questions ranging from the level of pain to the degree of discomfort during swallowing, with an arbitrary scale between 0 = no symptoms and 4 = very important symptoms.

Statistical Analysis

Data were processed using Graph Pad Prism, version 6.0f (Mac, San Diego, CA, USA) and are indicated as mean values ± standard deviation (SD). The significance of differences between measures in pre- *vs.* post-treatment in-group A (§), pre- *vs.* post-treatment in-group B (*), post-treatment group A *vs.* post-treatment group B (†) was compared using one-way ANOVA. A two-tailed p-value ≤ 0.05 was utilized throughout as criterion of statistical significance.

Results

A significant decrease in serum TSH levels was noted in patients of group B (MI-Se) compared prior and after treatment ($p \leq 0.001$), showing at baseline 4.22 ± 0.6 μIU/ml and 3.26 ± 0.89 μIU/ml over 6 months treatment. The decrement in controls pre- *vs.* post-treatment was not significant (4.32 ± 0.86 μIU/ml and 4.23 ± 0.89 μIU/ml). However, significance was observed comparing TSH values between group A and B post-treatment ($p \leq 0.001$) (Table I; Figure I).

The concentrations of fT_4 significantly increased in treated patients, from 0.93 ± 0.15 ng/dl

Table I. Overall results of laboratory parameters: serum TSH levels, fT4, fT3, TPOAb TgAb, and Subjective Symptomatology (SS) pre- and post-treatment with Se (group A) or with MI plus Se (group B) over a period of 6 months

Tests	Group A		Group B	
	Pre-treatment	Post-treatment	Pre-treament	Post-treatment
TSH μIU/ml	4.32 ± 0.86	4.23 ± 0.89	4.22 ± 0.6	3.26 ± 0.89***,†††
fT4 ng/dl	0.9 ± 0.25	0.95 ± 0.25	0.93 ± 0.15	1.06 ± 0.14 ***,†††
fT3 pg/ml	2.73 ± 0.35	2.81 ± 0.12	2.71 ± 0.44	2.83 ± 0.32
TPOAb IU/ml	820.13 ± 513.99	724.51 ± 524.98	733.7 ± 485.8	614.4 ± 472.0*,†
TgAb IU/ml	415.43 ± 275.89	422.03 ± 256.74	355 ± 220.5	298.8 ± 216.6**
SS	4.6 ± 0.63	2.75 ± 0.62§§§	4.71 ± 0.83	2.42 ± 0.81***,††

Statistical differences: group A pre- *vs.* post-treatment (§), group B pre- *vs.* post-treatment (*), group A *vs.* group B post-treatment (†). p-value: §≤ 0.05; §§≤ 0.01; §§§≤ 0.001. p-value: * ≤ 0.05; **≤ 0.01; ***≤ 0.001. p-value: †≤ 0.05; ††≤ 0.01; †††≤ 0.001.

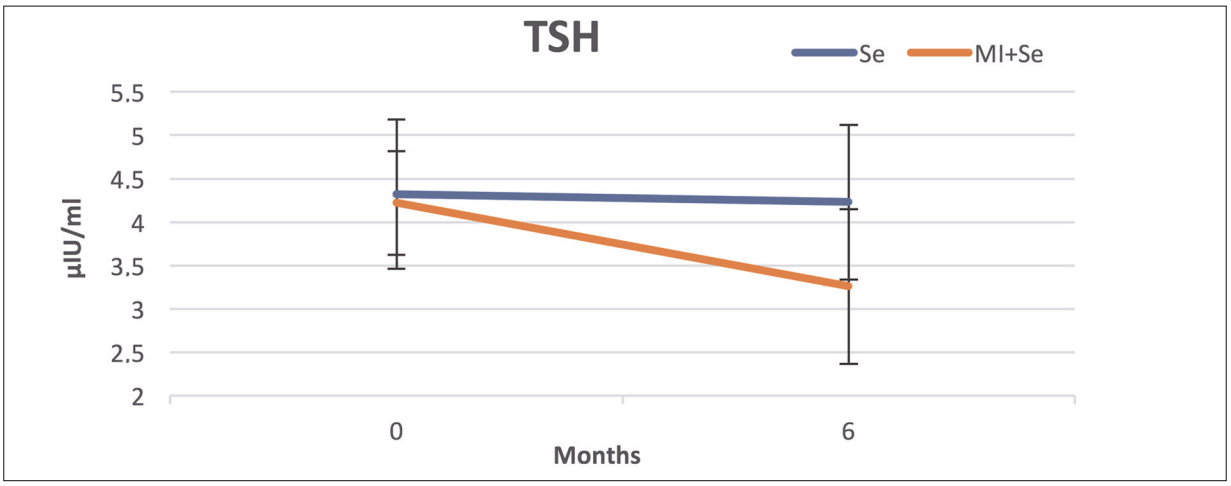

Figure 1. TSH levels. Results of TSH values at baseline and after treatment in both groups (Se and MI+Se). A reduction of TSH levels was observed after treatment in MI+Se group. Values are shown as mean ± SD.

at baseline to 1.06 ± 0.14 ng/dl after six months administration of MI-Se ($p \leq 0.001$). Between pre- and post- treatment, controls showed not statistical significance in fT_4 values (0.9 ± 0.25 ng/dl and 0.95 ± 0.25 ng/dl, respectively), whereas significance was seen in fT_4 titers inter-groups after 6 months therapy ($p \leq 0.001$) (Table I).

The mean fT_3 concentrations were approximately identical in both groups and statistical significance was not observed in any of the analysis of variance within groups, as well as between groups (group A pre-treatment 2.73 ± 0.35 pg/ml and post-treatment 2.81 ± 0.12 pg/ml; group B pre-treatment 2.71 ± 0.44 pg/ml and post-treatment 2.83 ± 0.32 pg/ml) (Table I).

At study entry, group B had a mean TgAb titer of 355.0 ± 220.5 IU/ml and at the end of the study 298.8 ± 216.6 IU/ml showing a not significant reduction. A slight, but not significant increase in TgAb titer was seen in controls after 6 months of L-selenomethionine administration, displaying as basal TgAb levels 415.43 ± 275.89 IU/ml and post-L-selenomethionine supplementation 422.03 ± 256.74 IU/ml. Variance between treated subjects *vs.* controls resulted significant ($p \leq 0.05$) (Table I). There was a significant decrease in mean TPOAb concentrations in group B (from 733.7 ± 485.8 IU/ml to 614.4 ± 472.0 IU/ml, ($p \leq 0.05$). The mean values of serum TPOAb concentrations in group A decreased from 820.13 ± 513.99 IU/ml to 724.51 ± 524.98 IU/ml, but the decrement was not significant. Significance was observed in the comparison between values obtained after six months administration in treated *vs.* controls ($p \leq 0.05$) (Table I).

A significant improvement of SS was reached among all patients. The questionnaire score showed a lowering from 4.71 ± 0.83 at baseline to 2.42 ± 0.81 post-treatment in combined MI-Se patients ($p \leq 0.001$), and from 4.6 ± 0.63 at beginning to 2.75 ± 0.62 at the end of the study in Se-patients ($p \leq 0.001$). One-way ANOVA revealed a significant variance between group A and group B after treatment ($p \leq 0.01$) (Table I).

In both groups at baseline plasma selenium levels were not different (132.4 ± 13.5 µg/l group A; 135.2 ± 14.8 µg/l group B). After 6 months treatment, selenium values were significantly augmented in each group compared to baseline (243.8 ± 20.2 µg/l, $p \leq 0.001$ and 234.6 ± 17.3 µg/l, $p \leq 0.001$). At baseline there were no differences between the two groups in the concentration of MI, whereas after treatment, a significant increase of plasma MI levels was observed only in the combined therapy group (27.3 ± 5.1 µmol/l *vs.* 42.2 ± 5.7 µmol/l, $p \leq 0.01$).

The SPV of the inferior thyroid artery was mainly reduced in patients treated with MI-Se, therefore showing a minimization of the inflammation (Data not shown).

Discussion

Our study confirms that oral administration of MI plus Se for over a period of 6 months significantly reduces serum TSH levels in Hashimoto's patients with subclinical hypothyroidism and raises thyroid hormones concentration. The changes in TPOAb and TgAb have been defined

as secondary outcomes of the study and, as expected, a lower TPOAb concentration was identified in both groups after treatment, whereas only in MI-Se group a reduction of TgAb titer was observed. Besides, a significant SS amelioration was observed in all patients of each group.

TSH is a very sensitive marker of thyroid function and, in milder forms of hypothyroidism like SH, it moderately increases, whereas thyroid hormones stay still in the normal range. As a matter of fact, the most important finding of this study was the reduction of TSH levels only in patients treated with combined MI-Se, but not in those treated only with Se. This might be interpreted as a crucial role of MI; indeed, its beneficial effect can be explained by the inositol biological function in the TSH signaling[21,26], regulation of iodination[23,27] and increased sensitivity of thyrocytes to TSH[20]. TSH and its receptor constitute the main regulatory pathway of thyroid; its signaling is quite complex involving two-second messengers, cyclic AMP and MI. Precisely, in human thyrocytes TSH activates the cAMP cascade and the Ca^{2+} phosphatidyl-inositol phosphate cascade (PIP_2)[29]. MI, found in the cell membranes, is implicated in cell growth, lipid synthesis, cell cytogenesis and morphogenesis; in particular, growth and differentiation of thyroid epithelial cells are mediated by the stimulation of the cAMP cascade, which requires the receptor activation by TSH and also the activation of the Gs alpha subunit complex. Moreover, MI is actually known to be involved in one of the first steps of thyroid hormone production. Therefore, as inositol modulates H_2O_2-mediated iodination and hypothyroidism can be caused by an impairment of inositol-depended TSH signaling branch (TSH resistance)[29], we speculate that, by increasing the amount of MI, the TSH sensitivity can be improved. The increase in thyroid hormones concentration was observed in both groups, but it was significantly higher in patients receiving the combined MI plus Se, thereby possibly due to the MI effect in regulating H_2O_2-mediated iodination. As a secondary outcome of this study the concentration of the antibodies TPOAb and TgAb was analyzed, and, as expected, a significant decrease of TPOAb in both groups was seen. In contrast to TPOAb levels, TgAb concentrations decreased in MI-Se-treated group, but increased slightly whilst not significantly in the control group. However, plasma TPOAb concentrations are specific for AIT and TgAb are less relevant for the pathogenesis, as well as the diagnosis of AIT. These results are in agreement with our previous findings[20], where, in the group treated with combined MI-Se, TPOAb decreased by 44% and TgAb by 48%. Reduction of TPOAb and TgAb in the present study was less relevant than in the preceding one; this may be due to the extended antibodies range at baseline.

Previous clinical trials[16,30,31] have reported a reduction of TPOAb levels after 6 months of treatment with L-selenomethionine demonstrating its valuable contribution in lowering TPOAb and ergo improving clinical conditions in AIT patients. L-selenomethionine is the most common oral form of Se supplementation, along with sodium selenite. Among all mechanisms regulating thyroid function, selenium-dependent enzymes exert a particular influence[32,33]. It has been demonstrated that Se deficiency, leads to glutathione peroxidase (GPx) inactivity, which may contribute to oxidative harm for thyroid cells and induction of thyroid damage and fibrosis[34]. Turker et al[35] have shown that TPOAb suppression in patients with AIT requires doses higher than 100 μg of L-selenomethionine per day to maximize GPx activities. These data are basically in agreement with ours, obtained by the daily administration of L-selenomethionine, even if we used a lower amount.

It has been shown that low Se intake is associated with a significantly greater incidence of negative mood states and depression[36,37]. Although mild thyroid failure is often asymptomatic, nearly 30% of patients with this disorder may have many debilitating symptoms such as mood-related problems, fatigue and muscle weakness, correlated to a change in the quality of life, that suggest a thyroid hormone deficiency. Therefore, in our study SS was also considered, including a questionnaire to evaluate changes in the quality of life and related symptoms over the trial period. Patients in both groups receiving either supplementation of Se alone or combined with MI reported significantly better wellbeing, which supports earlier findings[16,38]. This might be explained by the crucial Se activity in the brain and its association with senility and cognitive decline[39,40]. Nevertheless, patients treated with both MI and Se scored a better SS result, inducing an assumption that the combined elements might be more efficacious in improving the quality of life in hypothyroid patients. In fact, also inositol has shown to have therapeutic effects in the spectrum of illnesses responsive to serotonin selective reuptake inhibitors such as depression, panic and obsessive-compulsive disorder[41-43].

Furthermore, inositol has been shown to play a critical role in the etiology and treatment of polycystic ovary syndrome (PCOS)[44]. Indeed, several studies have reported the effectiveness of inositols, mainly the two stereoisomers MI and D-chiro-inositol (DCI), in improving the pathological conditions associated with PCOS[45-48]. In fact, although they exert different metabolic functions, both molecules are mediators of insulin action inside the cell[49]. As mentioned above, these two molecules have been shown to be involved in the signaling-transduction cascade of insulin and they exert important actions in the control of glucose homeostasis[49,50]. It has been established that insulin resistance and the consequent hyperinsulinaemia are relevant factors associated with the typical clinical signs and hormonal disorders of PCOS. MI and DCI administration, primarily in the physiological plasma ratio (40:1), has been proven to be an optimal approach for the treatment of PCOS disorders, by improving insulin resistance, serum androgen levels and many features of the metabolic syndrome[45,47]. It has also been shown by Paul et al[51] that MI and/or DCI work synergically in their metabolic actions and with other insulin sensitizing drugs and/or nutraceuticals. Moreover, the 2013 Florence International Consensus Conference on Myo- and D-chiro-inositol in obstetrics and gynecology has drawn attention to the use of inositols, according to their role in oocyte and spermatozoa development, in assisted reproductive technologies (ART)[48].

Another hot topic, that lately has been debated, concerns the relationship between AIT and heart and blood vessel diseases. Many studies consistently highlight the increased risks of coronary illnesses and cardiovascular mortality associated with SH[55,57], although the trend of higher risk is perceived in patients with TSH levels of 10 μIU/ml or greater[52]. Furthermore, thyroid hormones regulate heart rate and metabolism and decreased serum fT_3 and fT_4 levels can lead to a number of complications such as higher blood pressure, altered endothelial function, low heart rate, increased the stiffness of blood vessel walls and augmented heart strain[58]. Therefore, in our study, we have shown not only that administration of combined MI-Se decreases TSH levels but also significantly increases serum T_3 and T_4 concentration. The role of thyroid hormones in patients with coronary heart diseases is not completely understood, but they could protect indirectly the cardiac muscle by reducing the workload and oxygen consumption. We might then suppose that combination of MI-Se can also be a valuable aid in preventing cardiac complications in patients with AIT.

Conclusions

In this study, we wanted to highlight the beneficial effect of combined MI and Se, in improving clinical conditions of Hashimoto with subclinical hypothyroidism's patients having TSH level between 3.0 and 6.0 μIU/ml. Though L-T_4 efficacy is well recognized as a treatment when the TSH level is above 10 μIU/ml[52-54,59,60], it is still disputed for patients with serum TSH levels lower than 10 μIU/ml[13,55,56].

The present trial corroborates our previous results[20], confirming that the combined treatment, MI plus Se, is effective in significantly reducing the TSH and autoantibodies values in AIT, thus improving the well-being of patients and restoring the euthyroid state in Hashimoto's subjects with SH.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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