Double-Blind, Placebo-Controlled, Randomized Trial of Selenium in Graves Hyperthyroidism

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Context: Supplemental selenium (Se) may affect the clinical course of Graves disease (GD).

Objective: Evaluate efficacy of add-on Se on medical treatment in GD.

Design: Double-blind, placebo-controlled, randomized supplementation trial.

Setting: Academic endocrine outpatient clinic.

Patients: Seventy untreated hyperthyroid patients with GD.

Intervention: Additionally to methimazole (MMI), patients received for 24 weeks either sodium selenite 300 μg/d po or placebo. MMI was discontinued at 24 weeks in euthyroid patients.

Main Outcome Measures: Response rate (week 24), recurrence rate (week 36), and safety.

Results: A response was registered in 25 of 31 patients (80%) and in 27 of 33 (82%) at week 24 [odds ratio (OR) 0.93; 95% confidence interval (CI), 0.26 to 3.25; $P = 0.904$] in the Se (+MMI) and placebo (+MMI) groups, respectively. During a 12-week follow-up, 11 of 23 (48%) and 12 of 27 (44%) relapsed (OR 1.13; 95% CI, 0.29 to 2.66; $P = 0.81$) in the Se and placebo groups, respectively. Serum concentrations of Se and selenoprotein P were unrelated to response or recurrence rates. At week 36, 12 of 29 (41%) and 15 of 33 (45%) were responders and still in remission in the Se and placebo groups, respectively (OR 0.85; 95% CI, 0.31 to 2.32; $P = 0.80$). Serum levels of free triiodothyronine/free tetraiodothyronine, thyroid-stimulating hormone receptor antibody, prevalence of moderate to severe Graves orbitopathy, thyroid volume, and MMI starting dose were significantly lower in responders than in nonresponders. A total of 56 and 63 adverse events occurred in the Se and placebo groups, respectively ($P = 0.164$), whereas only one drug-related side effect (2.9%) was noted in 35 patients on placebo + MMI.

Conclusions: Supplemental Se did not affect response or recurrence rates in GD. ***(J Clin Endocrinol Metab* 102: 4333–4341, 2017)**

The essential trace element selenium (Se) is involved in thyroid hormone synthesis, metabolism, and action (1, 2). Deficiency of Se acts in concert with iodine (I) deficiency to impair thyroid metabolism and modifies the response to prophylactic I (3). Severe Se deficiency reduces the activity of selenocysteine-dependent enzymes (selenoproteins) including the iodothyronine deiodinases, thioredoxin reductases (TXNRD), and glutathione peroxidases (GPXs) and thereby impairs regular thyroid hormone metabolism in I-deficient populations (4, 5).

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Abbreviations: AE, adverse event; ATD, antithyroid drug; CI, confidence interval; fT3, free triiodothyronine; fT4, free tetraiodothyronine; GD, Graves disease; GO, Graves orbitopathy; GPX, glutathione peroxidase; I, iodine; MMI, methimazole; OR, odds ratio; ROS, reactive oxygen species; SD, standard deviation; Se, selenium; SELENOP, selenoprotein P; TPO-Ab, thyroid peroxidase antibody; TSH, thyroid-stimulating hormone; TSH-R-Ab, thyroid-stimulating hormone receptor antibody; TXNRD, thioredoxin reductase.

The normal thyroid gland retains high Se concentrations even under conditions of inadequate Se supply (6), similar to the brain (7). Adequate Se nutrition supports efficient thyroid hormone synthesis and metabolism and protects the thyroid gland from damage by excessive iodide exposure (8). In regions of combined severe I and Se deficiency, normalization of I supply is mandatory before initiation of Se supplementation to prevent further thyroid damage and hypothyroidism (9, 10). Severe Se deficiency impairs thyroid GPX and TXNRD expression and causes excessive peroxide concentrations in the endocrine gland (11). Besides affecting thyroid selenoproteins, Se status has an important immunomodulatory effect (12, 13). Cell-mediated immunity and B cell function depend on a sufficiently high Se supply. This dependence is related to several Se- and selenoprotein-dependent biochemical pathways implicated in correct protein folding, adequate calcium flux, and an efficient response to oxidative stress (14). Se deficiency also results in an increased rate of necrotic thyroid cells and promotes the invasion of macrophages. In several intervention studies, supplemental Se reduced the thyroid peroxidase antibody (TPO-Ab) levels, also improving thyroid gland echostructure (15). A few studies reported on accelerated recovery of hypothyroid patients upon Se supplementation (16), and importantly, on preventive effects on postpartum thyroid dysfunction and permanent hypothyroidism (13).

In Graves disease (GD), the balance between intracellular and extracellular oxidants and antioxidants is disturbed (17). Despite an increase in intracellular enzymes implicated in antioxidative defense (18), there is an overall decrease in GPX activity (19, 20) and other protective enzymes (superoxide dismutase, catalase) or redox-relevant molecules (vitamin E, coenzyme Q10) in GD. Consequently, the potential benefits of Se supplementation in GD were explored in pilot trials. Treatment with methimazole (MMI) alone vs MMI and a set combination of antioxidants (vitamin E, vitamin C, beta carotene, and Se at 60 μg/d) in subjects presenting GD was tested in Croatia (21), a country where nutritional Se levels are among the lowest in Europe. Plasma Se concentrations increased in subjects receiving Se, and erythrocyte GPX activity increased in both groups but significantly more in the MMI and antioxidant group. Furthermore, euthyroid status was attained more rapidly in the group receiving MMI and the antioxidant supplement. Plasma Se concentrations were also compared in patients with GD after discontinuation of antithyroid drugs (ATDs) (22). Although no differences were observed, Se levels were higher in patients in remission and correlated inversely to thyroid-stimulating hormone receptor antibodies (TSH-R-Abs). Finally, three small studies on Se in GD reported inconsistent effects of supplemental Se on biochemical and mental markers (*e.g.*, nervousness, irritability, insomnia, tremor) of hyperthyroidism (23–25).

Because the overall results from pilot studies provide an ambiguous picture, we performed a double-blind, placebo-controlled, randomized supplementation trial to assess the safety and efficacy of adjuvant Se intake on clinical course, response, and recurrence rates, and on serological parameters of GD. ATD efficacy was compared with the therapeutic effects of ATD combined with supplemental Se.

Methods

Patients and randomization

The prospective, randomized, double-blind, placebo-controlled clinical trial of Se in patients with GD (EUDRACT number 2010-022840-19) was approved by the Ethical Committee of the Johannes Gutenberg University, and written consent was obtained from all patients. A total of 70 consecutive, eligible, untreated hyperthyroid patients with GD were recruited at the endocrine outpatient clinic of the Johannes Gutenberg University Medical Center. GD was defined as positive serum levels of TSH-R-Ab, suppressed baseline TSH, elevated free triiodothyronine (fT3) or free tetraiodothyronine (fT4), and increased vascularization on thyroid imaging ("thyroid inferno"), with or without orbital disease. Patients were randomly assigned as follows: they received either MMI + placebo or MMI + Se, as sodium selenite 300 μg/d po for 24 weeks. The starting MMI daily dose was either 10 or 20 mg, depending on the severity of hyperthyroidism (below or above a threefold increased serum fT4 level). A dose titration was done at each visit according to the fT4 and fT3 serum levels. Se and placebo were not packaged in the same blisters as MMI; 10 mg of MMI was in a separate blister. The plan was to discontinue MMI therapy at 24 weeks in patients who were euthyroid. The matching placebo was given as dose-equivalent, identical-appearing tablets. The tablets were provided in patient packs containing five packages with 40 tablets each. The tablets were sealed in blister cards containing 10 tablets. Patient packs, packages, and blister cards were labeled with a unique patient number, corresponding to the randomization list.

A computer program generated a list and randomly allocated a patient number to one of the two treatment arms, starting with 001. Those numbers were also printed on the patient packs, packages, and blister cards, containing Se or placebo tablets, depending on the assigned number. If a patient fulfilled the inclusion criteria in the absence of any exclusion criteria and was enrolled (Supplemental Table 1), the investigator assigned a patient number to this patient, starting with 001, followed by 002 for the next patient. The patient number and patient identification data (name, date of birth) were recorded on a patient identification list at the site. The investigator dispensed the assigned packages to the patient. The patient was dispensed a supply sufficient for the interval between each study visit. Every dispensation and return of trial drugs was recorded on the drug accounting log at the site. Patients and site staff were blinded to identity of the treatment

group. Trial monitors and trial coordinator at the contract research organization were also blinded and did not have access to randomization information.

Primary and secondary outcomes

Efficacy

Patients were evaluated at baseline and 4, 12, 24, and 36 weeks after starting treatment. Clinical examination was performed by an investigator blind to treatment. Primary outcomes were the response rate at week 24 and the remission/recurrence rate at week 36. Response to treatment was defined as biochemical euthyroidism with thyroid-related hormones fT3, fT4, and TSH within the normal range. Relapse or recurrence was defined as increase of fT3 or fT4 above the normal range with a suppressed serum TSH.

Safety

Secondary outcomes were safety and tolerability of Se and MMI. Adverse events (AEs) were coded according to the standardized Medical Dictionary for Regulatory Activities (26) as recommended by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (27). AEs were analyzed for alternative causes and judged for relatedness to intake of Se (side effects). AEs were followed up until a stable outcome could be documented or until the patient was lost to follow-up. Seriousness of AEs was determined according to seriousness criteria defined in the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use E6 guideline for clinical practice (28). Patients exiting the study because of side effects or worsening of hyperthyroidism were kept in the primary analysis. White blood cell counts and liver enzymes were measured at weeks 0, 4, 12, and 24.

Serology

Blood sampling (thyroid-related hormones and autoantibodies) was performed at weeks 0, 4, 12, 24, and 36. Serum concentrations of TSH, fT3, fT4, thyroglobulin antibody, and TPO-Ab were evaluated with electrochemiluminescent immunoassays (CLIA, Abbott, Wiesbaden, Germany) according to the manufacturer's instructions. For diagnosis of GD, serum samples were measured for thyroid-binding inhibitory immunoglobulins with the Cobas e411 analyzer, Elecsys (Roche Diagnostics, Mannheim, Germany).

Selenium and selenoprotein P measurements

Collected blood samples were kept frozen until the end of database lock, and concentration analysis was done subsequently. Se and selenoprotein P (SELENOP) serum levels were measured as described (29). Briefly, SELENOP concentrations were determined in duplicates from 20 μL of sample *via* a commercial colorimetric enzyme immunoassay (Selenotest®; ICI Immunochemical Intelligence GmbH, Berlin, Germany). Total Se concentrations were measured by total reflection x-ray fluorescence. Briefly, 10 μL of sample was spiked with an internal gallium standard (Sigma-Aldrich, Taufkirchen, Germany) and applied to polished quartz glass carriers. After drying at 37°C for 3 hours, x-ray evoked fluorescence spectra were recorded and analyzed. Interassay and intra-assay coefficients of variation were <15%, as determined with a commercial human reference serum sample (Seronorm; SERO AS, Billingstad, Norway). Duplicate determinations varying by >20% of SELENOP or Se concentrations were reanalyzed. Analyses were conducted by personnel blinded to study details and patient characteristics in a laboratory remote from the contract research organization.

Statistical analysis

The trial was designed to detect a difference in response proportions between 40% (placebo + MMI) and 80% (Se + MMI) by two-sided χ^2 test at type 1 error of 0.05 and power 0.80 with 23 evaluable patients per group. In anticipation of dropout, sample size was set to 35 patients per group. Statistical analysis was performed with the SPSS/PC software package for MS Windows (released 2013, IBM SPSS Statistics for Windows, version 22.0; IBM Corp., Armonk, NY). Distribution of metric variables was characterized by reporting medians and quartiles or means and standard deviations (SDs) when appropriate. For dichotomous variables, absolute and relative frequencies were shown. Treatment groups were statistically compared by means of the Mann–Whitney *U* test (metric variables), Fisher exact test, or two-sided χ^2 test (dichotomous variables). To examine whether a change occurred between times 0, 4, 12, 24, and 36 weeks, within patients of a treatment group, the Wilcoxon signed rank test was applied for metric variables and the McNemar test for dichotomous variables. Primary outcome response at week 24 was defined for each patient who appeared at the week 24 visit. Recurrence was defined as biochemical hyperthyroidism at week 36 for responders to treatment at week 24. Response proportions, proportions of patients relapsing among responders, and proportion of patients responding and not relapsing among all evaluable patients were compared by Fisher exact test and odds ratio (OR) with 95% confidence interval (CI). All reported *P* values are two-sided. Significance level was set at 0.05.

Results

Demographic data

A total of 70 hyperthyroid patients with untreated GD were randomly enrolled in the two-arm treatment trial (Fig. 1). The demographic, clinical, and serological data are shown in Table 1. There were no relevant or significant differences between the study groups pertaining to sex, age, race, smoking rate, presence of other autoimmune endocrine or nonendocrine disorders, prevalence of Graves orbitopathy (GO), number of patients with previous ATD treatment, or serum levels of thyroid-related hormones and autoantibodies.

Primary and secondary outcomes

The intent-to-treat population included all randomly assigned patients receiving at least one tablet and was used to analyze primary and secondary efficacy outcomes. The safety population included all dosed patients and was used for all safety analyses.

Efficacy

A response to medical treatment and biochemical euthyroidism was registered in 25 of 31 patients (80%)

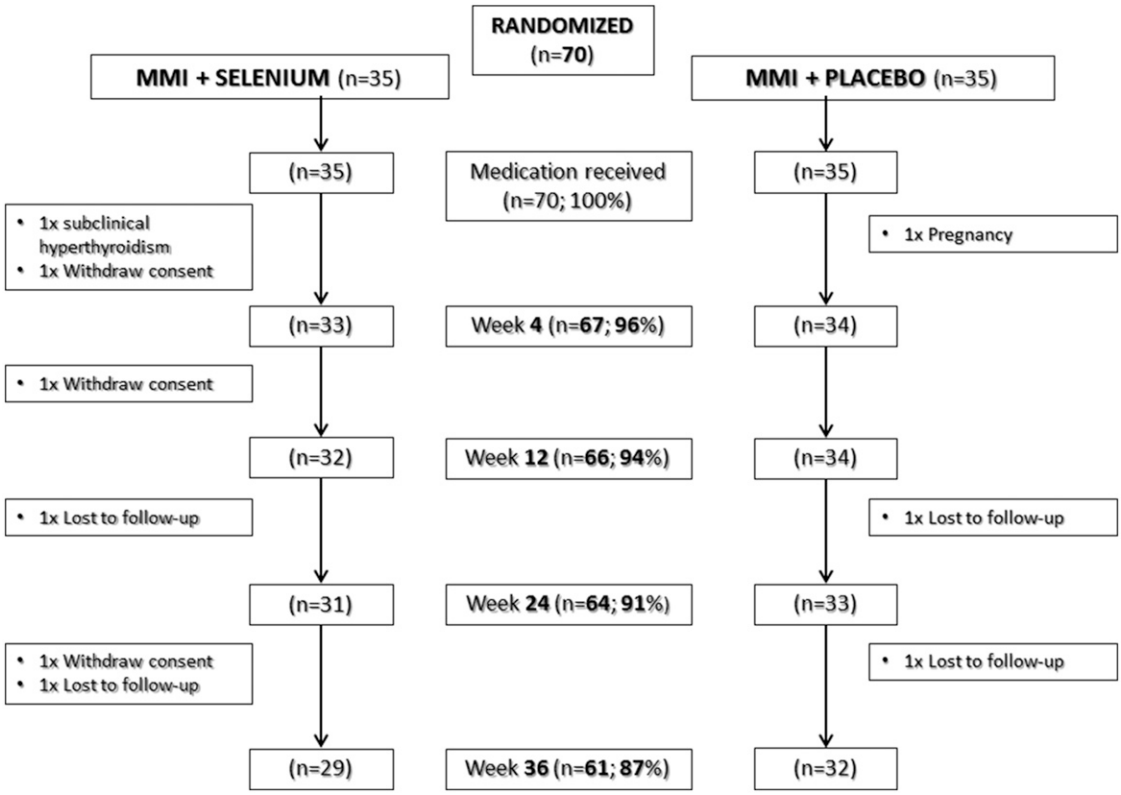

Figure 1. Randomization chart. All patients were on ADs.

and in 27 of 33 (82%) at week 24, OR 0.93 (95% CI, 0.26 to 3.25; $P = 0.90$) in the Se (+MMI) and placebo (+MMI) groups, respectively. The plan was to discontinue MMI therapy at 24 weeks in patients who were euthyroid. The serum level of fT3 (mean ± SD 7.9 pg/mL ± 4.2 vs 16 pg/mL ± 10.9, $P < 0.001$), TSH-R-Ab (median, 25/75 percentile 5.4 IU/L, 3.4/14.7 vs 27.3 IU/L, 6.3/65.2, $P = 0.001$), TPO-Ab (median 25/75 percentile 136 IU/L, 3/666 vs 761 IU/L, 229/1000, $P = 0.018$), and prevalence of mild GO (14 vs 1, $P = 0.041$) were markedly different between responders and nonresponders. In contrast, age, sex, smoking, onset of GD, previous treatment with ATD, and the presence of thyroid nodules did not affect the response rate.

During the 12-week follow-up, 11 of 23 (48%) and 12 of 27 (44%) relapsed (OR 1.13; 95% CI, 0.29 to 2.66; $P = 0.81$) in the Se and placebo groups, respectively. Therefore, at week 36, 12, of 29 (41%) and 15 of 33 (45%) were responders and still in remission in the Se and placebo groups, respectively (OR 0.85; 95% CI, 0.31 to 2.32, $P = 0.80$). The serum concentrations of Se and/or SELENOP did neither increase the response nor decrease the recurrence rate.

Safety

A total of 119 reversible and controlled minor to moderate AEs were registered in 70 patients without suspected unexpected serious side effects (Supplemental Table 2). A total of 56 AEs and 63 AEs occurred in the Se (+MMI) and placebo (+MMI) groups, respectively ($P = 0.164$). In comparison, only one drug-related side effect was noted in 1 of 35 (2.9%) patients on placebo + MMI. The patients were monitored closely and immediately given further therapy when recurrence was identified, and they were monitored in the 3 months between stopping MMI (week 24) and eventually declaring them to have failed to achieve a remission (week 36). There were no AEs associated with recurrent hyperthyroidism after stopping MMI (*e.g.,* hospitalization for severe thyrotoxicosis, cardiac arrhythmia).

Course of the individual parameters and correlations with Se status

Se and SELENOP serum levels significantly increased during treatment in the Se group in contrast to placebo (Table 2). Both biomarkers of Se status responded positively to supplemental Se in the Se group (Supplemental Fig. 1) and remained largely unaltered in the placebo group. Supplemental Se increased serum SELENOP concentrations almost linearly, indicating that the subjects were suboptimally supplied for full SELENOP expression. Serum levels of SELENOP correlated with serum Se levels ($r = 0.791$, $P < 0.001$) and serum TSH ($r = 0.37$, $P = 0.003$) but negatively with serum fT3 ($r = -0.29$, $P = 0.026$) and serum TPO-Ab levels ($r = -0.32$, $P = 0.013$). Serum Se levels negatively correlated with serum TPO-Ab ($r = -0.28$, $P = 0.027$).

Table 1. Demographic, Clinical, and Serological Data of All Study Patients

	Selenium + MMI	Placebo + MMI	P[a]
N	35	35	
Age, y, mean (SD)	44.5 (13.8)	44.5 (13.4)	1.0
Height, m, mean (SD)	1.69 (0.091)	1.69 (0.066)	0.988
Weight, kg, mean (SD)	68.8 (11.2)	71.8 (11.7)	0.270
Systolic blood pressure, mm Hg, mean (SD)	118.0 (11.5)	120.4 (14.2)	0.435
Diastolic blood pressure, mm Hg, mean (SD)	71.9 (6.1)	73.0 (9.8)	0.569
Heart rate, beats per min, mean (SD)	76.7 (13.7)	79.1 (11.4)	0.418
White, N (%)	34 (97.1%)	34 (97.1%)	1.000
Asian, N (%)	1 (2.9%)	1 (2.9%)	
Female, N (%)	28 (80%)	26 (74.3%)	0.569
Menopause, N (%)	12 (34.3%)	9 (25.7%)	0.434
Smoker, N (%)	16 (45.7%)	17 (48.6%)	0.811
Ex-smoker, N (%)	7 (20%)	9 (25.7%)	0.569
Passive smoker, N (%)	7 (20%)	5 (14.3%)	0.562
Pack years, median (25; 75 percentile)	10 (4; 31)	9 (5; 25)	0.673
Allergic, N (%)	12 (34.3%)	15 (42.9%)	0.461
No other autoimmune disease, N (%)	32 (91.4%)	30 (85.7%)	0.300
Type 1 diabetes, N (%)	0	2 (5.7%)	0.151
Vitiligo, N (%)	1 (2.9%)	0	
Psoriasis vulgaris, N (%)	1 (2.9%)	1 (2.9%)	
Neurodermitis, N (%)	0	3 (8.6%)	
Sjögren syndrome, N (%)	1 (2.9%)	0	
Crohn disease, N (%)	0	1 (2.9%)	
GO, N (%)	20 (54%)	17 (46%)	0.473
Mild, N (%)	10 (27%)	10 (27%)	
Moderate to severe, N (%)	10 (27%)	7 (19%)	
Dermopathy	0	0	1.000
Age at diagnosis of GD, y, mean (SD)	42.2 (15.2)	42.5 (14.5)	0.923
Pretreated, N (%)	29 (82.9%)	26 (74.3%)	0.382
Previous treatment cycles, mean (SD)	1.29 (1.0)	1.09 (0.82)	0.359
MMI, mg, median (25; 75 percentile)	10 (5; 10)	10 (5; 20)	0.822
Goiter palpable, N (%)	17 (48.6%)	23 (65.7%)	0.147
Thyroid volume, mL, median (25; 75 percentile)	19.1 (14.2; 29.5)	20.1 (16.1; 34.9)	0.414
Thyroid nodes, N (%)	4 (11.4%)	2 (5.7%)	0.393
Echogenicity			0.205
Normal, N (%)	8 (22.9%)	4 (11.4%)	
Reduced, N (%)	27 (77.1%)	31 (88.6%)	
Vascularization			0.495
Increased, N (%)	4 (11.4%)	6 (17.1%)	
Strongly increased, N (%)	31 (88.6%)	29 (82.9%)	
Thyroid structure			0.078
Homogeneous, N (%)	2 (5.7%)	0	
Inhomogeneous, N (%)	32 (94.3%)	35 (100%)	
fT3, 1.7–3.7 pg/mL, mean (SD)	8.9 (4.2)	9.7 (5.01)	0.640
fT4, 0.7–1.5 ng/mL, mean (SD)	2.0 (0.4)	2.2 (0.6)	0.457
TSH, 0.4–4.9 mU/L, median (25; 75 percentile)	0.01 (0.01; 0.01)	0.01 (0.01; 0.01)	0.536
TSH-R-Ab, <1.8 IU/L, median (25; 75 percentile)	6.4 (4.4; 17.1)	6 (3.2; 19)	0.660
TPO-Ab, <6 IU/mL, median (25; 75 percentile)	190 (3; 760)	330 (21; 868)	0.772
Tg-Ab, <4.1 IU/mL, median (25; 75 percentile)	8.1 (2.2; 45.9)	5.4 (1.7; 45.3)	0.647
Thyroglobulin, 3.5–77 ng/L, median (25; 75 percentile)	66.7 (12.8; 366.8)	86.25 (10.2; 196.8)	1.000
Selenium, μg/L, mean (SD)	109.2 (24.8)	115 (29.3)	0.384
SELENOP, mg/L (SD)	3.8 (0.8)	3.7 (1.1)	0.828

Abbreviation: Tg-Ab, thyroglobulin antibody.

[a]Fisher's exact test or two-sided χ^2 test for categorical variables, Mann-Whitney *U* test for quantitative variables.

Serum values of the thyroid-related hormones were within the normal range at week 24, without significant differences between the two groups. Serum concentrations of the thyroid-related autoantibodies significantly dropped within the groups without significant differences between the groups. Median thyroid volume ($P = 0.027$), number ($P = 0.016$) of thyroid glands with increased vascularization ("thyroid inferno"), number of thyroids with hypoechoic imaging ($P = 0.022$), and inhomogeneous ultrasound structure ($P = 0.003$) decreased significantly in the placebo group only; however, no significant changes were noted pertaining to ultrasound parameters between the groups.

Table 2. Serological Results

	Selenium + MMI					Placebo + MMI				
	Week 0	Week 4	Week 12	Week 24	Week 36	Week 0	Week 4	Week 12	Week 24	Week 36
Selenium, μg/L	109 (25)	189 (209)	209 (261)	160 (51)	117 (43)	115 (29)	107 (34)	107 (27)	111 (27)	110 (26)
Vs baseline		$P = 0.030$	$P = 0.035$	$P < 0.001$	$P = 0.395$		$P = 0.036$	$P = 0.024$	$P = 0.574$	$P = 0.373$
Selenium vs placebo	$P = 0.384$	$P = 0.026$	$P < 0.001$	$P < 0.001$	$P = 0.406$					
SELENOP, mg/L	3.8 (0.8)	5.2 (1.1)	5.1 (1.1)	5.1 (1.3)	3.3 (0.9)	3.7 (1.1)	3.6 (1.0)	3.6 (1.0)	3.6 (1.0)	2.9 (0.8)
Vs baseline		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.008$		$P = 0.248$	$P = 0.413$	$P = 0.509$	$P < 0.001$
Selenium vs placebo	$P = 0.828$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.082$					
fT4, 0.7–1.5 ng/dL	2.0 (0.6)	1.1 (0.3)	1.1 (0.4)	1.0 (0.3)	1.3 (0.6)	2.2 (0.8)	1.2 (0.4)	1.1 (0.4)	1.1 (0.5)	1.3 (0.4)
Vs baseline		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Selenium vs placebo	$P = 0.457$	$P = 0.328$	$P = 0.517$	$P = 0.633$	$P = 0.651$					
fT3, 1.7–3.7 pg/mL	8.9 (6.2)	3.6 (1.6)	4.2 (3.9)	3.6 (3.2)	4.7 (3.2)	9.7 (7.0)	3.8 (2.0)	3.7 (2.5)	3.8 (4.8)	4.5 (5.2)
Vs baseline		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Selenium vs placebo	$P = 0.640$	$P = 0.660$	$P = 0.551$	$P < 0.852$	$P = 0.848$					
TSH, 0.4–4.9 mU/L	0.01	0.01	0.03	0.83	0.05	0.01	0.01	0.45	1.38	0.12
Median, 25/75 *P*	001/0.01	0.01/0.11	0.01/1.01	0.02/1.88	0.01/0.78	0.01/0.01	0.01/0.01	0.01/1.78	0.14/2.46	0.01/0.56
Vs baseline		$P = 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$		$P = 0.016$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Selenium vs placebo	$P = 0.536$	$P = 0.055$	$P = 0.244$	$P = 0.287$	$P = 0.887$					
TSH-R-Ab, <1.8 IU/L	6.4	9.4	8.6	5.3	4.5	6.0	5.9	5.15	3.0	2.65
Median, 25/75 *P*	4.4/17.1	4.25/23.3	3.55/17.7	2.2/19.4	1.6/12.7	3.2/19	2.7/16.3	2.1/12.1	1.7/8.7	0.7/6.3
Vs baseline		$P = 0.118$	$P = 0.645$	$P = 0.003$	$P = 0.001$		$P = 0.198$	$P = 0.014$	$P < 0.001$	$P < 0.001$
Selenium vs placebo	$P = 0.660$	$P = 0.139$	$P = 0.077$	$P = 0.107$	$P = 0.161$					
TPO-Ab, <6 IU/mL	190	171	103.5	95	97	330	310	301	145	97
Median, 25/75 *P*	3/760	3/761	3/721	3/720	3/487	21/868	14.5/840	7.5/857	10.5/596	8/484
Vs baseline		$P = 0.586$	$P = 0.407$	$P = 0.237$	$P = 0.015$		$P = 0.465$	$P = 0.426$	$P = 0.077$	$P = 0.042$
Selenium vs placebo	$P = 0.772$	$P = 0.666$	$P = 0.582$	$P = 0.664$	$P = 0.856$					
Tg-Ab, <4.1 IU/mL	8.1	7	5.3	4.1	3.9	5.4	5.8	4.5	3.3	3.2
25/75 *P*	2.2/46	2.3/42	1.9/66.6	1.6/45.5	1.8/22.4	1.7/45.3	1.7/41.7	1.6/36.6	1.7/30.2	1.4/31.6
Vs baseline		$P = 0.381$	$P = 0.592$	$P = 0.136$	$P = 0.036$		$P = 0.079$	$P = 0.035$	$P = 0.001$	$P = 0.101$
Selenium vs placebo	$P = 0.647$	$P = 0.860$	$P = 0.807$	$P = 0.920$	$P = 0.965$					
Thyroid volume, mL	19.1			17.7		20.1			16.7	
25/75 *P*	14.2/29.5			10.7/32		16.1/35			13.6/29.3	
Vs baseline				$P = 0.252$					$P = 0.027$	
Selenium vs placebo	$P = 0.414$			$P = 0.883$						

Values are mean (SD) unless otherwise specified.

Abbreviation: Tg-Ab, thyroglobulin antibody.

Patients with response to treatment vs those with nonresponse or relapse

At week 36, 27/61 (44%) patients responded to ATD treatment and were still in remission 12 weeks after stopping therapy, and 34/61 (56%) were either nonresponders at week 24 or rapidly relapsed during follow-up. These groups were compared with respect to all baseline characteristics shown in Table 1. Significant differences between the groups are shown in Supplemental Table 3. Compared with responders with sustained remission, the prevalence of GO and of clinically moderate to severe GO in particular, serum fT3/fT4 levels, starting ATD dose, thyroid volume, and prevalence of goiter were markedly higher in nonresponders and those who rapidly relapsed during follow-up.

After completion of this trial, study patients were again seen at week 50, 6 months after dropping ATD treatment. Results of thyroid function evaluation were similar to those at week 36 (data not shown), thus confirming the week 36 findings in both groups.

Discussion

This double-blind, placebo-controlled, randomized, prospective clinical trial in a large number of therapy-naive patients showed no obvious advantage of adding a relevant daily dose of Se in the form of sodium selenite to MMI in the medical treatment of GD. The response rate, number of patients with normal thyroid-related hormones, and decrease of serum levels of thyroid-related autoantibodies at week 24, as well as the high relapse rate after completion of ATD therapy, were comparable between trial arms. Although a significant increase of the serum levels of Se and SELENOP was noted in the Se group, no significant differences were registered between the two groups pertaining to efficacy and the clinical course of thyroid disease. The Se and SELENOP serum levels were not associated with the response or recurrence rate in GD. In this study, SELENOP was the more meaningful biomarker of Se status as compared with total serum Se concentrations, because it correlated significantly with both thyroid-related hormones and thyroid autoantibodies. AEs occurred in both groups, partly disease-related, but Se-related side effects were not observed during administration of 300 μg daily of sodium selenite or during follow-up.

Serum levels of Se and SELENOP measured sequentially before, during, and after treatment showed no Se deficiency in most patients. This finding could explain the null effect of administering an add-on relevant daily dose of Se on the response and relapse rates of GD in this study.

Nevertheless, the positive relation of serum SELENOP and Se concentrations indicates that the patients were not fully Se replete, because SELENOP expression has been shown to reach a constant level once a sufficient Se intake is reached (30). The results obtained here support the notion that GD has some relation to the Se status because both total Se and SELENOP concentrations negatively correlated with serum TPO-Ab, and SELENOP positively correlated to serum TSH. However, although serum Se levels significantly increased in the Se (+MMI) group, the decrease of thyroid-related autoantibody levels was similar to the one observed in the placebo group, indicating a lack of adjuvant effect of supplemental Se on the ATD treatment. Our antibody data are comparable to those observed in the double-blind, placebo-controlled trial of the European Group on Graves' Orbitopathy (31) in patients with GO, which showed significant improvements in eye signs and quality of life in the Se group. However, ophthalmic improvement was not accompanied by a marked decrease in thyroid-related antibodies, and the Se and SELENOP serum levels were not documented. These missing Se levels and potential variant pathomechanisms between GD and GO may explain the different results.

To demonstrate a potential efficacy of Se on the course of GD, we have chosen a 6-month treatment period, allowing us to evaluate the recurrence rate after completion of ATD treatment. Achieving biochemical euthyroidism and normalization of thyroid-related hormone levels was not a medical challenge in the majority of patients; however, we were confronted by a high recurrence rate of ~50%, which was similar in both groups. Therefore, we not only divided our patients into responders and nonresponders at week 24 but also looked carefully at those who responded and further stayed in remission at weeks 36 and 50, trying to differentiate them via clinical and serological parameters from those who did not respond or rapidly relapsed within weeks. Here again, no significant differences were registered between patients on Se and those on placebo. However, we cannot rule out the possibility that continuing Se supplementation after cessation of ATD treatment would have affected the relapse rate. The results are in line with the negative findings in a Se-sufficient Italian population in one small study (32), where selenomethionine (166 μg Se/d) was used as a supplement over a course of 90 days. However, our results contrast with the beneficial effects reported from a small Scandinavian study using selenized yeast as supplement (200 μg Se/d) over a course of 9 months, where the authors observed improved biochemical control of thyroid dysfunction under a block and replace regimen (23). Similarly, positive effects of supplemental Se have been reported from a small study with a higher remission rate and improvement of laboratory parameters of hyperthyroidism in patients with recurrent GD receiving supplemental Se for 6 months (25). These divergent results indicate that a direct comparison of the currently available Se supplementation studies in GD is hampered by the differences in Se compound, dosage, and time of supplementation chosen, by the baseline Se status of the patients, which is not always determined, and by the inclusion and exclusion criteria applied.

As previously stated, oxidative stress plays a role in GD, which creates reactive oxygen species (ROS) in peripheral tissues (19). ROS may cause thyrocyte damage, resulting in increased antigen exposure to the immune system and worsening of autoimmunity. Furthermore, ROS may be responsible for tissue damage in peripheral tissues, thereby contributing to the clinical manifestations of GD (33). Pertaining to the antioxidative and potential immunomodulation effect of Se, supplementation with Se has been associated with decreased lymphocyte and monocyte cytokine release (34) as well as thyroid antibody levels, albeit to variable extents in different studies and different patient groups (35, 36). TPO-Ab reduction may result through elevated intrathyroid Se levels achieved, with consequently reduced oxidative damage via increased expression of the GPX and improvement of redox status in the thyrocyte through increasing activity of TXNRD. However, thyroid Se concentrations and SELENOP expression levels are not accessible for analysis and may be independent from circulating Se and SELENOP concentrations. Furthermore, Se supplementation reduces production of regulatory CD4+ CD25+ T cells by upregulating FOXP3 expression and increasing the percentage of regulatory T cells (37), which act as a powerful brake on immune responses, restoring them to normal levels. The increasing serum Se level from a mean low baseline level toward the normal range is paralleled by an increase in erythrocyte GPX1 activity.

Several factors may be interpreted as limitations of this trial: the short treatment period of 6 months and follow-up interval of 3 months, the unanswered but potential likelihood that a longer duration of Se may have affected the outcomes, the lack of documentation of Se-related effects on quality of life, the lack of data on parameters of oxidative stress or damage, the lack of assessment of I levels, the modest number of randomly assigned patients in each group, and the possibility that results from a similar study in a different geographic area with different endemic Se concentrations could give divergent results. Nevertheless, this is the largest randomized, prospective trial with a double-blind, placebo-controlled design and both a clinical informative follow-up and precisely documented, sequential

measurements of Se and SELENOP serum levels in patients with GD under adjuvant supplemental Se treatment.

Conclusions

This carefully conducted, double-blind, placebo-controlled supplementation trial of an add-on relevant daily dose of Se did not positively affect the clinical course and the serological parameters of Se-sufficient, hyperthyroid patients with GD.

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