# Serum Selenium Levels in Patients with Remission and Relapse of Graves' Disease

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**Abstract:** ***Objective:*** Selenium (Se) in the form of selenocysteine is an essential component of the family of the detoxifying enzymes glutathione peroxidase (Gpx) and of the iodothyronine selenodeiodinases that catalyze the extrathyroidal production of tri-iodothyronine (T(3)). Thus, Se deficiency may seriously influence the generation of free radicals, the conversion of thyroxine (T(4)) to T(3) and a thyroidal autoimmune process. The aim of this study was to investigate whether serum Se levels may influence the outcome of Graves' disease (GD).

***Design And Methods:*** 83 patients (77 women, 6 men) with active GD were retrospectively analyzed (mean age 40,0 years). Twenty-four GD patients went into remission and were euthyroid during follow-up (median follow-up: 20.1 months), whereas 59 patients did not go into remission or developed relapse over the following 24 months. TSH receptor autoantibodies (TRAb) were measured using the second generation assay on the basis of human TSH receptor. Se levels were determined at the first visit in our outpatient clinic and were correlated with TRAb levels and clinical outcome of these patients.

***Results:*** Median TRAb levels in the group of remission were significantly ($p<0.0001$) lower than TRAb values in the relapse group (2.1 as compared to 8.6 IU/l). By comparing mean serum Se levels in the remission and relapse group no significant differences were seen (73.0 *vs*. 71.7 ug/l). Detailed analyses of both groups of patients, however, revealed that highest serum Se levels (>120 ug/l) were seen in the remission group, indicating a positive effect of Se levels on the outcome of GD. In addition, we also compared these results with TRAb levels of these patients. We could show that TRAb levels and serum Se values were positively correlated in the relapse group, whereas a negative correlation of both parameters were seen in the remission group, supporting the idea of a positive effect of Se on thyroidal autoimmune process.

***Conclusion:*** Our data indicate that high serum Se levels (>120 ug/l) may influence the outcome of GD. This is important, as Se administration trials in GD, which are under discussion need to be performed with Se supplementation at higher dosages than used in autoimmune thyroiditis.

**Key Words:** Serum selenium levels, Graves' disease, remission, relapse, TSH receptor autoantibodies, autoimmune thyroiditis.

## INTRODUCTION

Selenium (Se) is a trace mineral and as selenocysteine, the 21st amino acid, forms the central component of selenoproteins. Thirty-five of selenoproteins have been identified so far, most of which have enzymatic function [1]. Selenium also has a pivotal influence on the immune system possibly through its central role in lymphocyte function [2] and selenium deficiency is associated with markedly lower resistance to viral infections [3]. Held accountable for this is a significantly higher level of oxidative stress in Se deficiency as Se acts as an important antioxidant presenting its redox function e.g. in the glutathione peroxidases. A lack of Se consequently leads to insufficient reduction of hydrogen peroxide as well as phospholipid hydroperoxides eventually resulting in cell death [4]. It is this precise capacity that plays a central role in the thyroid gland: glutathione peroxidase (GPX1) alongside thioredoxin reductase (TR1) as well as the deiodinases (D1-4) are all involved in thyroid hormone synthesis. Whilst the deiodinases catalyze T3 formation from T4 [5], GPX1 and TR1 have a protective effect on thyrocytes through the reduction of $H_2O_2$ accumulating during hormone synthesis [6,7]. In Se deficiency, protection from free radicals is inadequate and, therefore, the apoptotic response is increased [4], an effect which also links Se to the development of cancer and cardiovascular disease [8].

The links between Se deficiency, altered immune function and increased thyroidal apoptotic response prompted studies in humans to examine if Se supplementation can modify autoantibody production in patients with chronic autoimmune thyroiditis. Double-blind, randomized, placebo-controlled trials using daily supplements of 200µg selenite produced a significant decline in anti-TPO-antibody (TPO-Ab) concentrations accompanied in some patients by an improved ultrasound echogenicity of the thyroid gland [9,10]. This effect of Se on TPO-Ab concentration has been demonstrated both in an area of Germany with marginal dietary iodine and Se intakes [9] and in an area around Athens /

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Greece where iodine and Se intakes were close to requirement [11]. In these studies Se supplements had no significant effect on the concentration of thyroglobulin antibodies (Tg-Ab) or the concentration of TSH or thyroid hormone concentrations. The mechanism by which Se exerts effects on TPO-Ab production is likely to be due to the ability of high doses of Se to modify the inflammatory and immune responses (reviewed in [12]). In Graves' disease (GD), it has also been reported that Se supplementation may positively modify the short-term course of disease in terms of a faster achievement of euthyroidism while treating with methimazole and some antioxidants including selenium [13]. This has, however, only been demonstrated in a small number of GD patients without a long-term follow-up. Nonetheless, in an autoimmune mouse model, for instance, it has been shown that Se supplementation decreases TNF-α expression and increases interleukin-10 expression in lymphocytes [14,15] which represents a potential mechanism for modifying the inflammatory activity in GD.

Based on these results it could also be assumed that serum Se may correlate with the natural course of GD, e.g. that normal (or elevated) Se levels may correlate with remission of GD, whereas Se deficiency may result in aggravation of the disease and relapse of GD. $H_2O_2$ induced alteration of thyrocytes may prompt increased antigen presentation and, consequently, an intensified autoimmune response. The aim of this study was to explore this concept. All results were compared with Se values in non-autoimmune thyroid disease (goiter patients) and with patients with Hashimoto's thyroiditis.

## MATERIAL AND METHODS

### Patients

In order to explore the coherence of serum selenium levels and GD, 83 patients (77 female, 6 male) with active GD were included. The criteria for GD were based on initially documented hyperthyroidism with or without ophthalmopathy and positivity within the TSH receptor antibody assay (see below). The criteria establishing hyperthyroidism were clinical symptoms, increased serum concentrations of free thyroxine ($T_4$), increased free triiodothyronine ($T_3$) and decreased TSH.

All GD patients were on anti-thyroid drug (ATD) treatment (thiamazole or carbimazole 2.5 to 20 mg) at the time of their first visit to our outpatient clinic (in median 5.1 months after initial diagnosis). The median duration of received ATD treatment was 5.1 months as well. Out of the 83 patients, 59 patients (mean age 40.0 yrs., range 15-70; 13 men, 46 women) did not go into remission or relapsed within 24 months. Twenty-four patients developed clinical remission (mean age 52 yrs., range 21-72; 3 men, 21 women). The median remission time of all of these patients was 20.1 months. Patients with autoimmune thyroiditis (n = 50) and non-autoimmune thyroid disease (goiter patients; n = 50) have been included for comparison of data.

### Detection of Serum Selenium Levels

Serum Se levels were determined in triplicates with a graphite furnace atomic absorption spectrometer with the Zeeman background correction (Perkin Elmer Analyst 600) by using a standard addition method. Samples were compared with the standard curve by linear least-squares fit analysis. The LOQ (limit of quantification) for Se was 10 µg/L (0,13 µmol/L). Within run and run-to run coefficients of variations (CV) for Se were 6 % and 8 % respectively.

### TSH Receptor Antibody Assay

TRAb were detected by the DYNOtest® TRAK human (B.R.A.H.M.S. AG, Hennigsdorf /Berlin, Germany). This assay uses the human recombinant TSH receptor (TSH-R) on coated tubes, human antibodies for standard material, and expresses the results in international units (IU/L) based on a WHO standard. Based on our own clinical evaluation of this assay, values below 1.0 IU/l were defined as negative and values above 1.5 IU/l are positive [16]. Patients with initial TRAb units within the grey zone of 1.0 – 1.5 IU/l who developed higher scores during follow-up were considered as positive. All samples were measured within one assay run according to the manufacturer´s instructions.

### Thyroid Functional Tests

The serum concentrations of TSH (reference range: 0.3 – 3.5 µU/ml, lower detection limit: 0.01 µU/ml), free $T_4$ (normal range: 9.1 – 19.1 pg/ml) and free $T_3$ (2.6 – 5.1 ng/l) were measured by time resolved electrochemiluminiscence assay (Roche).

### Statistical Analysis

All data are presented as means of triplicate determinations. Comparison was done by non-parametric assays (Mann-Whitney test, Spearman Correlation) calculated with Prism computer software (GraphPad Software Inc., San Diego, CA).

## RESULTS

The mean serum Se level of all GD patients was 72.1 ug/l (± 22.1). This value was not significantly different from serum Se levels in patients with autoimmune thyroiditis (75.3 ± 16.4) or in non-autoimmune goiter patients (72.7 ± 16.0) (Fig. **1**). Detailled analyses of both GD groups with and without remission revealed Se serum values of 73.0 ug/l ± 31.1 and 71.7 ug/l ± 17.5, respectively. No significant differences between both groups could be seen either (Fig. **2**).

We next looked on whether serum Se levels might correlate with serum TRAb values. Using a cut-off limit of 1.0 IU/l, all 83 patients were positive for TRAb (median: 11.1 IU/l; range: 1.1 – 81.5 IU/l) at the time of their first visit to our outpatient clinic. Plotting all TRAb levels against serum Se levels of all GD patients a slightly negative correlation (r = -0.03) became apparent (Fig. **3A**). Detailled analyses of both groups, however, revealed a more pronounced positive correlation in the relapse group (r = 0.03) and a moderately negative correlation in the remission group (r = -0.23) (Figs. **3A** and **3B**). In other words, these results indicate that increasing serum selenium levels may contribute to a diminished autoimmune process in GD leading to lower TRAb serum levels in the remission group. These differences did, however, not reach statistical signifance.

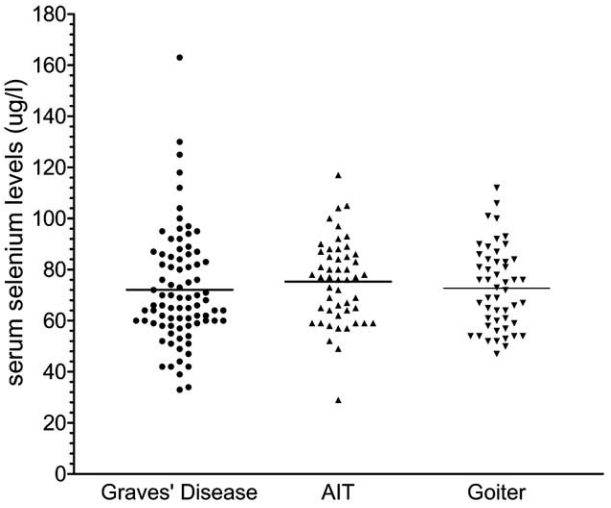


**Fig. (1).** Distribution of serum selenium levels in autoimmune and non-autoimmune thyroid disease:

Mean serum selenium levels were not significantly different in patients with Graves' disease compared to patients with autoimmune thyroiditis and non-autoimmune goiter.

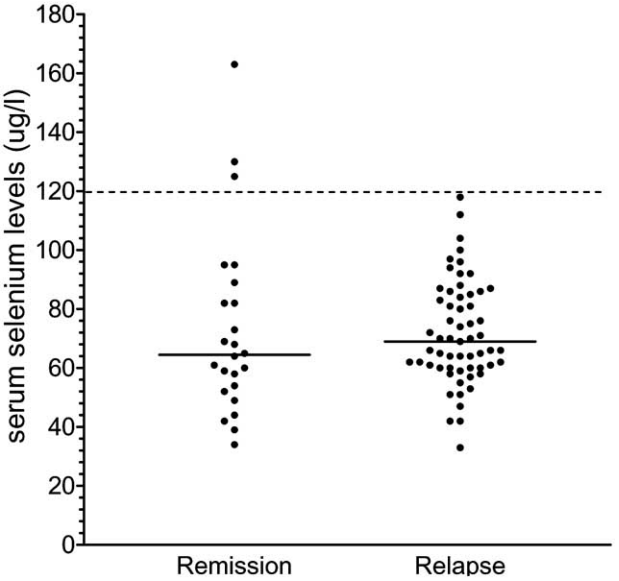


**Fig. (2).** Distribution of serum selenium levels:

Mean serum selenium levels in Graves' disease (GD) patients with remission and relapse were not significantly different. Most important, however, highest values (>120 ug/l) were seen in three patients with remission of GD indicating that high serum selenium levels may correlate with remission of GD.

We next compared different cut-offs of serum Se levels in both groups of patients in order to identify a minimum serum Se level for a positive prediction of remission. When applying a higher cut-off limit of 100 µg/l three patients reached remission, whereas also another 3 patients relapsed (Fig. **2**). Using a much higher threshold of 120 µg/l we only identified patients to be part of the remission group. The serum Se values in these patients were 125.0 µg/l, 130.0 µg/l and 163.0 µg/l. No single patient with serum-selenium-levels above 120 µg/l could be identified in the relapse group which accounts for a lack of statistical calculations of these findings.

## DISCUSSION

In this retrospective study we aimed to examine whether there is an interdependence between serum selenium (Se) levels and the autoimmune process, including the outcome of Graves' disease (GD). We could demonstrate a more positive correlation between serum Se levels and TSH receptor autoantibodies (TRAb) in the relapse group, whereas the remission group revealed a more pronounced negative correlation. Even though these differences did not reach statistical significance they suggest that serum Se levels may have an impact on the autoimmune process in general. For detailed analyses, we also compared the clinical outcome of all patients depending on the magnitude of serum Se concentrations. Using a threshold of 120 µg/l we saw that all patients experienced stable remission. From our collected data we can, therefore, conclude that high serum selenium levels (> 120 µg/l) may have an influence on the outcome of GD. Keeping this in mind whilst designing follow-up trials in GD patients will be important since higher supplemental dosages of selenium than those used before in patients with autoimmune thyroiditis have to be considered. Gärtner and coworkers for instance, used 200 µg sodium selenite per day for the treatment of patients with autoimmune thyroiditis leading to serum Se levels around 1.09 µmol/l (84.8 µg/l). To reach the suggested serum levels of above 120 µg/l in patients with GD, application of an about three time higher minimum dose of sodium selenite (~600 µg per day) over a defined period of time will presumably be necessary. Below that threshold no significant clinical aspect in terms of outcome of GD may be expected. Whether lower doses of sodium selenite administration will have impact on non-thyroidal manifestations of GD such as endocrine ophthalmopathy can not be concluded from our data.

Influential features of the effect that selenium exerts on thyroid function and the role it plays in autoimmune processes, have been identified before: the most evident being its key role as an indispensable component of thyroid hormones deiodinases (D1 and D2), thioredoxin reductase (TR1) and glutathionperoxidases (GPX), the latter representing by far the most important antioxidant involved in normal thyrocyte function [17,18]. Under pathophysiological conditions such as destructive Hashimoto's thyroiditis or GD it might be postulated that $H_2O_2$-induced alteration of thyrocytes may prompt increased antigen presentation and, consequently, an intensified and prolonged autoimmune response. As Se acts as an important antioxidant presenting its redox function e.g. in the glutathione peroxidases it might also be postulated that the positive effects of Se supplementation on the outcome of autoimmune thyroid diseases are due to this pathway.

As Selenoprotein P has been suggested to function as an important, possibly the most important extracellular Se supply it may not be enough to monitor serum Se levels on their own for an accurate quantification of Se actually obtainable to the individual at any given point in time [19]. Selenoprotein P is, however, difficult to estimate and so far no commercially available assay has been established. Another influence on Se availability to the thyroid in particular may be the varying distribution of the trace mineral between differ-

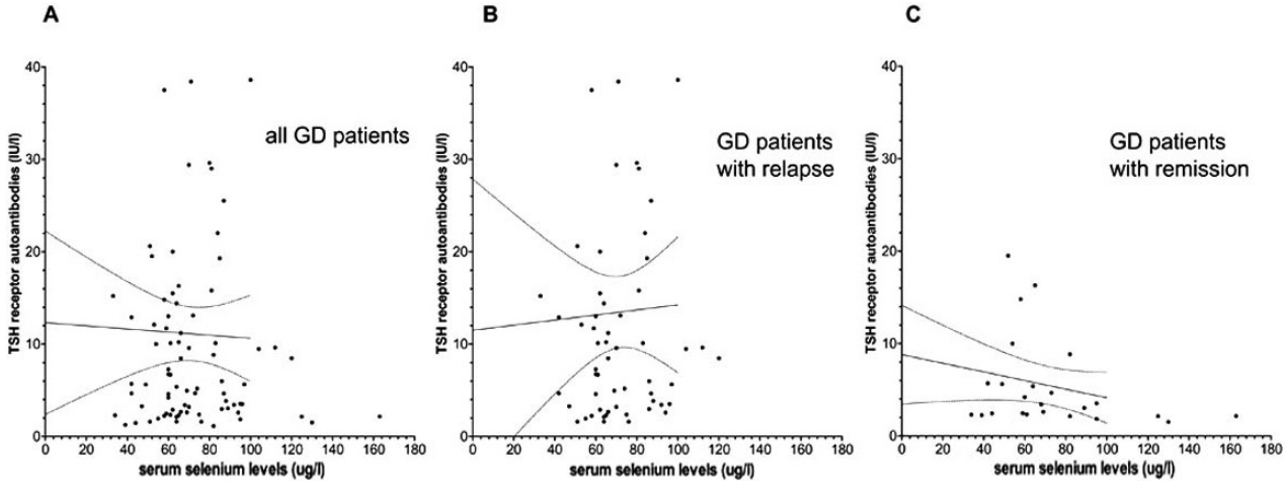


**Fig. (3).** Correlation between serum selenium levels and TSH receptor autoantibodiies:

By plotting TSH receptor autoantibody levels of all Graves' disease (GD) patients against serum selenium (Se) levels a slightly negative correlation (r = -0.03) became apparent (Figure 3A). Detailed analyses of both GD groups, however, revealed a more pronounced positive correlation in the relapse group (r = 0.03) and a moderately negative correlation in the remission group (r = -0.23). These differences, however, did not reach statistical signifancy.

ent organs depending on the state of either Se depletion or abundance. It has been stated before that in endocrine tissues Se content is naturally high and that selenoprotein expression is maintained even in Se deficiency [20,21].

Furthermore, indicative features of the progression of GD and the inflammatory activity like changes in thyroid volume and ultrasound echogenicity as well as clinical manifestations and indicators of thyroid function like thyroid hormone serum levels ought to provide additional parameters of thyroid function for the measurement and assessment of the possible influence of high serum selenium levels on the course of GD in upcoming studies on the subject. At that time point, our results already suggest that different serum selenium levels may have an impact on the outcome of GD.

## ACKNOWLEDGEMENTS

We thank all colleagues of the Department of Endocrinology, Diabetes and Rheumatology for their help and support in the performance of the study.

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**Received: 08 June, 2006** **Revised: 13 July, 2006** **Accepted: 14 July, 2006**