

Relative selenium insufficiency is a risk factor for developing severe Graves' orbitopathy: a case-control study

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ABSTRACT

Background/aims Selenium (Se), an antioxidant agent, is effective in preventing mild Graves' orbitopathy (GO) deterioration. However, the significant risk of low serum Se concentration for GO progression has not been identified. Here, we aimed to investigate the risk of relative Se insufficiency and to identify its optimal cut-off value in the development of disease severity in patients with GO.

Methods Serum Se levels were prospectively measured in 100 consecutive patients with GO. The patients were classified into groups with mild and severe GO (logistic regression analysis outcome). A receiver operating characteristic (ROC) curve and the minimum p value corresponding to χ^2 statistics were analysed to select the optimal cut-off Se level for the diagnosis of severe orbitopathy.

Results Thirty-two patients (32%) had mild GO and 68 (68%) had severe GO. The ROC revealed a cut-off Se level of 93 µg/L. Se levels ≤93 µg/L were observed in 48.5% and 12.5% of the patients in the severe and mild (p<0.001) groups, respectively. The risk estimate (OR) for an Se level ≤93 µg/L was 8.14 (95% CI 2.39 to 27.75). It remained a significant risk factor after adjusting for age, sex, thyroid status, smoking status, thyroidectomy and radioactive iodine.

Conclusion Relative Se insufficiency (≤93 µg/L) is a potential risk factor for severe GO development. An evaluation of Se status is recommended in patients with GO for predicting disease progression and guiding supplementation therapy.

INTRODUCTION

Selenium (Se) is a substantially available trace element that plays a broad range of biological roles in human beings. It plays an important role in antioxidant and anti-inflammatory activities and aids in maintaining a healthy metabolism through its role in the production of active thyroid hormone.¹ It enters the human food chain through plants, seafood and Se-supplemented animal feed.² The Se status varies by country and correlates with intake. Se intake is low in Europe and some parts of China and is high in Venezuela, Canada, the USA and Japan.¹ The average serum Se levels reported in two different

Key messages

What is already known about this subject?

- ▶ Selenium (Se), an antioxidant agent, is effective in preventing mild Graves' orbitopathy (GO) deterioration. However, the risk of low serum Se concentration and its cut-off point value to predict GO progression have not been identified.

What is the new finding?

- ▶ Relative Se insufficiency (≤93 µg/L) is a potential risk factor for severe GO development.

How might these results change the focus of research or clinical practice?

- ▶ An evaluation of Se status is recommended in patients with GO for predicting disease progression and guiding supplementation therapy.
- ▶ Correcting Se insufficiency with supplementation may restore the pathological changes in the orbit caused by oxidative stress.

healthy Thai populations were 106.95 µg/L and 114.96 µg/L.^{3 4} In order to induce the nutritional functions of Se, it is incorporated into selenoproteins with an active centre consisting of selenocysteine. The highest concentration of Se is found in the thyroid gland in the form of glutathione peroxidase 3 (GPX3) that protects thyroid cells from hydrogen peroxide in thyrocytes and the follicular lumen.² Several systematic reviews and meta-analyses have revealed that Se supplementation could reduce thyroidperoxidase autoantibody concentrations in patients with autoimmune thyroiditis.⁵⁻⁷

Graves' orbitopathy (GO) is a multifactorial autoimmune disease. It is characterised by the initial reaction against autoantigens present in orbital fibroblasts and thyroid epithelial cells.⁸ Autoreactive lymphocytes infiltrate orbital tissues and initiate a cascade of inflammatory processes by releasing growth factors, cytokines and reactive oxygen species.⁹ The pathogenesis ultimately causes



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the proliferation of orbital fibroblasts that differentiate into adipocytes and myofibroblasts leading to an increased production of hydrophilic glycosaminoglycans.^{8,9} The volume expansion of orbital tissues leads to proptosis, diplopia, dry eye, optic neuropathy and facial disfigurement. Thyroid-stimulating hormone receptor (TSHR) and insulin-like growth factor-1 receptor autoantibodies have been described as being potentially related to the GO pathogenesis.¹⁰ The findings in a randomised control trial of sodium selenite treatment in patients with mild GO included a significantly improved quality of life, less eye involvement and a gradual progression of GO.¹¹ This evidence highlights the importance of Se in treating GO and the possibility that Se supplementation has an effect that reduces oxidative stress and can be used to correct Se deficiency. However, the Se status of patients with GO was not evaluated in the aforementioned study. Based on the results of a case-control study on the serum Se status of patients with Graves' disease with or without orbitopathy in an Australian population, it was concluded that patients with GO had lower serum Se levels than those with Graves' disease and that the mean Se levels decreased in parallel with the increasing severity of orbitopathy.¹² By contrast, Dehina *et al* reported on a lack of an association between Se and the severity or activity of GO in German patients.¹³ To our knowledge, there have been no studies in which Se was identified as an independent risk factor of GO. Therefore, in this study, we aimed to determine whether a relative serum Se deficiency may be a risk factor for the development of severe orbitopathy. In an attempt to identify the relative risk, we aimed to analyse the Se level cut-off point for the diagnosis of mild and severe GO.

MATERIALS AND METHODS

Patients diagnosed with GO were consecutively recruited from the oculoplastic clinic of Mettapracharak Eye Center, Thailand, from 2018 to 2020. Patients aged under 18 years and those with an eating disorder or poor intestinal absorption were excluded. Serum samples were collected from patients (N=100) before the initiation of Se replacement treatment. Serum Se was assessed by graphite furnace atomic absorption spectrophotometry. The study was conducted in accordance with the Declaration of Helsinki, and informed consent was obtained from all the included patients. The study procedures were approved by the Institutional Review Board of Mettapracharak Hospital. The patients' demographic data, including sex, age, current thyroid status, degree of proptosis, smoking status and treatment for GO, were analysed. Eye examinations were performed by ophthalmologists and confirmed by an oculoplastic surgeon with expertise in thyroid eye disease. The European Group of Graves' Orbitopathy (EUGOGO) case record form and the Color Atlas reference were used. The activity of the disease and soft tissue inflammation was graded using the clinical activity score (CAS) depending on the inflammatory signs within the orbit (pain, redness and swelling).

The CAS is the sum of the following items present: (1) spontaneous retrobulbar pain, (2) pain on eye movement, (3) redness of the eyelids, (4) redness of the conjunctiva, (5) swelling of the eyelids, (6) inflammation of the caruncle or plica and (7) conjunctival oedema. A CAS $\geq 3/7$ indicates active GO. Disease severity was determined based on the EUGOGO classification system, and patients were classified into mild, moderate to severe and very severe groups. Patients with mild GO have minor lid retraction (<2 mm), exophthalmos <3 mm, mild soft tissue involvement and transient or no diplopia. Patients with moderate to severe GO have lid retraction ≥ 2 mm, exophthalmos ≥ 3 mm, inconstant or constant diplopia. Patients with very severe GO have dysthyroid optic neuropathy and/or corneal breakdown. The patients in the moderate and severe groups were classified as having severe GO (case). The mild severity group included patients with mild GO (control).

Blood collection and serum Se determination

Blood samples were collected in clot blood tubes and centrifuged for Se analyses. The serum was separated within 3 hours and stored at -80°C for a biochemical analysis. The Se level was measured using graphite furnace atomic absorption spectrophotometry with the SpectrAA 600 system (Varian, Victoria, Australia) and a specific Se electrode discharge lamp.

Statistical analyses

Statistical analyses were performed using the SPSS V.18.0 software (SPSS, Chicago, Illinois, USA). Normality distribution of serum Se levels was evaluated using the Shapiro-Wilk normality test. As the Se levels in the mild and severe disease groups were normally distributed, Student's t-test was used to compare their means. The cut-off point of the Se level for the diagnosis of mild and severe GO was determined using a receiver operating characteristic (ROC) analysis and the minimum p value of the χ^2 approach as defined by Miller and Siegmund.¹⁴ We used the χ^2 test and Fisher's exact test to confirm the statistically significant differences between predicted and observed frequencies among the categorical variables. We used severe disease as the outcome for binary logistic regression. The independent variables included Se level, age, sex, thyroid status, smoking status, diabetes mellitus, thyroidectomy and radioactive iodine ablation.

RESULTS

Sixty-eight patients with severe GO and 32 controls with mild GO were recruited for participation in the study. [table 1](#) presents the demographic data and clinical characteristics of the patients. Sixty-two patients (62%) were woman and 38 (38%) were men. The mean age was 48.8 ± 12.2 years. Sixty-nine patients were in a euthyroid state (69%). The severe GO group exhibited features of dysthyroid optic neuropathy (10%) and moderate to severe disease (58%). Exophthalmos values were significantly greater in severe GO than in mild GO

Table 1 Demographic data of patients with GO

Variable	All	Mild GO (control)	Severe GO (case)	P value
Sex (female:male)	62:38	24:8	38:30	0.07
Age	48.8±12.2	47.38±14.4	49.41±11.2	0.44
Exophthalmos (mm)				
Right eye	18.1±3.5	17.2±3.1	18.6±3.6	0.04
Left eye	18.8±4.7	17.3±3.2	19.5±5.2	0.02
Euthyroid state (current status)	69	26	43	0.07
Clinical activity score (minimum– maximum)	0.4±0.9 (0–6)	0.2±0.6 (0–2)	0.4±1.1 (0–6)	0.19
Smoking	9	1	8	0.27
Diabetes	13	2	11	0.22
Treatment for GO				
Intravenous methylprednisolone	19	4	15	0.26
Oral steroid	12	1	11	0.10
Orbitotomy	20	5	15	0.45
Treatment for thyroid disease				
Antithyroid medication	54	16	38	0.58
Radioiodine	15	5	10	1.0
Thyroidectomy	5	1	4	1.0
Thyroxine	18	7	11	0.49

GO, Graves' orbitopathy.;

($p=0.04$, 0.02). As for the treatment of GO, 20% of the patients required orbitotomy, 19% received intravenous methylprednisolone and 12% received oral steroids. The mean serum Se level in the mild disease group ($106.14\pm18.24\mu\text{g/L}$) was higher than that in the severe disease group ($97.99\pm24.67\mu\text{g/L}$), with no statistically significant difference ($p=0.06$).

Figure 1 depicts the ROC curve for the use of Se levels in the prediction of severe orbitopathy. The true positive rate (sensitivity) is plotted as a function of the false-positive rate (1-specificity) for different cut-off points of the Se level. The 'C' point of the ROC curve depicts the optimal Se level with a low false-positive rate (0.1) and estimated sensitivity of 0.5. Table 2 presents the cut-off points of Se levels with their sensitivity and specificity values. A Se level of $93\mu\text{g/L}$ was chosen as the cut-off point at a specificity of 0.85 and sensitivity of 0.48.

Serum Se levels of equal to or less than $93\mu\text{g/L}$ were significantly associated with severe GO ($p<0.001$, OR 6.60, 95% CI 2.08 to 20.85) (table 3). These levels remained significantly associated with severe GO after adjusting for age, sex, thyroid status, diabetes, smoking status, thyroidectomy and radioiodine treatment in a multiple logistic regression analysis ($p=0.001$, OR 8.14, 95% CI 2.39 to 27.75) (table 4).

The odds of being men, having diabetes and smoking were approximately two times greater among patients with severe GO than among those with mild GO. The odds of having an abnormal thyroid status were approximately three times higher among patients with severe GO than among those with mild GO.

DISCUSSION

Given the role of oxidative stress in the GO pathogenesis, Se is believed to serve an important function in the course of this condition owing to its antioxidative properties. Several studies have indicated the dual effects of Se on

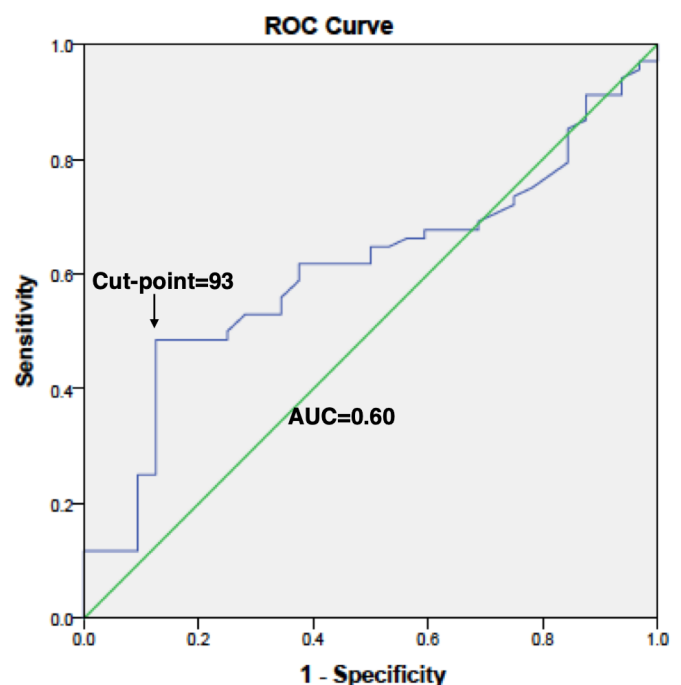


Figure 1 ROC curve for the use of selenium levels in the prediction of severe orbitopathy. ROC, receiver operating characteristic; AUC, area under the curve.

Table 2 The cut-points of selenium levels with their sensitivity and specificity values

Cut point	Specificity	Sensitivity
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86.00	0.87	0.33
88.00	0.87	0.38
89.50	0.87	0.39
90.50	0.87	0.41
91.50	0.87	0.42
92.56	0.87	0.48
93.56	0.85	0.48
95.00	0.79	0.48
96.50	0.75	0.48
97.50	0.75	0.50
98.10	0.72	0.52
98.50	0.69	0.52
98.89	0.65	0.52
...

orbital fibroblasts. It may prevent fibroblast proliferation and protect tissue from cytotoxic damage.⁹ The hypothesis that we tested was that relatively insufficient Se levels may be related to severe GO. This study is the first to indicate that a relatively insufficient Se level ($\leq 93 \mu\text{g/L}$) is an independent risk factor for the development of severe orbitopathy. Currently, there are no universal normal ranges of serum Se levels, and the Se status varies among different countries.¹⁵ Nevertheless, it may be possible to estimate the cut-off levels for Se concentrations for evaluating the sufficiency of Se status. The estimated cut-off concentration for preventing Keshan disease is $>21 \mu\text{g/L}$; for optimal activity of iodothyronine 5' deiodinase, it is $>65 \mu\text{g/L}$; for maximisation of plasma GPx and seleno-protein P, it is $>80\text{--}95 \mu\text{g/L}$; and for protection against some cancers, it is $>120 \mu\text{g/L}$.¹⁵ A study from France found that the deficient levels in adults were $\leq 59 \mu\text{g/L}$.¹⁶ Se deficiency $\leq 85 \mu\text{g/L}$ is associated with decreased survival in HIV- infected patients.¹⁷ Interestingly, we found that the cut-off level in our study is similar to values of $90 \mu\text{g/L}$ and $95 \mu\text{g/L}$ derived from studies from New Zealand,^{18,19} regarding plasma Se needed to achieve the full expression of GPx and corresponds with the study by Yang *et al*, in which plasma GPX was found to plateau at a whole-blood Se concentration of $89 \mu\text{g/L}$.¹⁵ Meanwhile,

it is lower than the average levels found in healthy Thai individuals ($106 \mu\text{g/L}$ and $114.96 \mu\text{g/L}$).^{3,4} In a Danish study, newly diagnosed Graves' disease, patients had lower Se concentrations than the controls. The authors attribute this finding to a link between Se deficiency and autoimmune thyroid disease.²⁰ In a study conducted in Germany, a significant negative correlation was found between serum Se levels and TSHR autoantibody concentrations.¹³ This emphasises the importance of correcting serum Se levels that are higher than the cut-off point in order to restore the pathological changes in the orbit resulting from oxidative stress. However, in contrast with our expectation, the means of the Se levels in the mild and severe groups were not significantly different from those in a Thai population with adequate Se intake. This outcome is similar to the result of a study from Germany¹³ but is contrary to that in a study by Khong JJ *et al* in Australian populations.¹² In the latter study, it was reported that the mean Se levels decreased in parallel with the increased severity of GO. However, the effect of GO severity on Se levels appeared to be marginal in their study ($1.10 \pm 0.19 \mu\text{M}$ in moderate to severe GO, $1.09 \pm 0.17 \mu\text{M}$ in very severe GO).

The EUGOGO published a randomised clinical trial on Se supplementation in 159 patients with mild GO who were treated with $200 \mu\text{g}$ of Se per day, 1200mg of pentoxifylline per day, or a placebo. The results showed a significant improvement in the overall ophthalmological outcomes and quality of life in the Se-treated patients compared with those in the placebo group.¹¹ This is the first study in which the benefit of treatment with Se was identified in GO. However, a limitation of the EUGOGO study is that baseline Se concentrations were not evaluated. Hence, there is no evidence regarding whether the effectiveness of Se supplementation in treating GO could be attributed to the baseline Se levels, which may have been quite different, as these levels vary by the country of the patients' residence.

Evidence regarding the immunostimulant effects of Se in humans is scarce. A study on elderly volunteers who received $400 \mu\text{g/day}$ of Se revealed a significant increase of 27% in the total T-cell count in these participants compared with those in the placebo group.²¹ A UK-based study on Se supplementation in adults with a low Se status commenced with supplementation of $50 \mu\text{g}$ or $100 \mu\text{g}$ of Se per day, following which the participants were vaccinated with a single oral dose of live attenuated poliovirus, and it was found that the virus clearance was quicker in

Table 3 Chi-square depicting selenium levels of $\leq 93 \mu\text{g/L}$ with the minimum *p*-value indicating a significant association with severe GO

Selenium levels	Disease	Severity	P value	OR	95% CI	
	Mild (N=32)	Severe (N=68)			Lower	Upper
$\leq 93 \mu\text{g/L}$, N (%)	4 (12.5%)	33 (48.5%)	<0.001	6.60	2.08	20.85
$>93 \mu\text{g/L}$, N (%)	28 (87.5%)	35 (51.5%)				

GO, Graves' orbitopathy.;

Table 4 Multiple logistic regression model for selenium levels of $\leq 93 \mu\text{g/L}$, which were significantly associated with severe disease after adjusting for demographic factors

Variables	Coefficient	SE	Wald	P-value	OR	95% CI for OR
Selenium $\leq 93 \mu\text{g/L}$	2.09	0.63	11.22	0.001	8.14	2.39 to 27.75
Age	0.001	0.02	0.004	0.95	1.00	0.96 to 1.04
Male sex	0.85	0.56	2.29	0.13	2.33	0.78 to 6.99
Abnormal thyroid status	1.18	0.58	4.14	0.04	3.24	1.04 to 10.04
Smoking	0.74	1.25	0.35	0.55	2.09	0.18 to 24.19
Thyroidectomy	0.65	1.29	0.25	0.62	1.91	0.15 to 23.88
Radioiodine	0.07	0.69	0.01	0.92	1.07	0.28 to 4.11
Diabetes	0.84	0.87	0.94	0.33	2.33	0.42 to 12.81

participants who received Se than in those who received a placebo.²²

The Se status can be evaluated by measuring the serum concentration of total Se or selenoproteins. To avoid overdosage in patients with baseline concentrations higher than $122 \mu\text{g/L}$, it is important to assess the Se status before commencing on supplementation.²³ Overdosage is associated with type 2 diabetes and malignancies.^{1,9} However, supplementation with $200 \mu\text{g/day}$ of sodium selenite over 6 months in patients with GO was not found to be associated with major side effects or adverse events in a European population.¹¹

There are some limitations to our study, including the lack of a healthy control group without orbitopathy; hence, a comparison of the Se level analyses between the case and control participants (nonorbitopathy) was not possible. The mean Se level in the mild orbitopathy group (as control) was equal to the average values in healthy Thai individuals. Further investigation of serum Se or selenoprotein levels in healthy individuals, those with Graves' disease without orbitopathy, and those with GO will generate more evidence on the effects of Se on the disease course.

The area under the curve (AUC) is a performance measurement for classifying patients with and without a disease based on various threshold settings. The AUC in this study represented a marginal (0.6) degree of separability; thus, in general, the serum Se model demonstrated a low performance in distinguishing between mild and severe GO. Since GO is a multifactorial disease, considering the Se level as a risk factor in combination with other factors may enhance prognosis prediction.

The generalisability of findings from this study might be limited by the potential variation in clinical GO evaluations performed by different examiners (reproducibility) and intrapersonal variation (repeatability). Hence, several approaches including the EUGOGO criteria, case record form and reference atlas were used to ensure the reliability of GO assessment. In addition, only one oculoplastic surgeon responded to the re-evaluation and confirmation of clinical findings before data were collected.

In conclusion, our study indicates that relatively insufficient serum Se ($\leq 93 \mu\text{g/L}$) is an independent risk factor for developing severe GO in a Thai study population with an optimal Se status. Correcting Se insufficiency with supplementation may restore the pathological changes in the orbit caused by oxidative stress. An evaluation of the Se status is recommended in patients with GO before commencing supplementation therapy.

Contributors ML conceived the study design, analysed the data and drafted the manuscript. UB and PT drafted the research proposal and collected the data. SS, ON and JK interpreted the data and revised the manuscript. KP participated in the research methodology, performed the statistical analyses, and coordinated the manuscript drafting. All authors have read and approved the final manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the Mettaphracharak Hospital Institutional Research Committee, and informed consent was obtained from all the individual participants included in the study.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article.

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