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Serum selenium status in Egyptians patients who had Graves' disease with and without ophthalmopathy

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Abstract

Selenium is efficient in reducing the progression of active Graves' orbitopathy and improving life quality. The impact of mending relative deficiency of selenium on improving Graves' orbitopathy is not known, due to the lack of previous measurement of baseline levels of selenium. The study object was to determine whether serum selenium levels are lower in patients with Graves' ophthalmopathy (GO) disease in comparison with those without ophthalmopathy. This prospective case control study was conducted between 2019 and 2021 at the endocrine and ophthalmology clinics, Ain Shams University, Cairo. The study included a total of 75 subjects, 50 patients with Graves' disease (GD) and 25 subjects as a control group. Of the GD patients, 25 had Graves' orbitopathy. Serum selenium concentrations were measured in each group. The mean level of serum selenium was significantly lower in patients with Graves' orbitopathy (16.6 ± 7.5 ng/ml) than in patients with Graves' disease (42.9 ± 8.2 ng/ml) (p< 0.001). Mean selenium levels were reduced with increasing severity of GO, as selenium level was 30-55 ng/ml in GD, 21-28 ng/ml in mild GO, 18-22 ng/ml in moderate GO and 5-16 ng/ml in severe GO (p< 0.001). In conclusion, serum selenium levels were lower in GO patients compared with GD patients in an Egyptian population. Low selenium levels may be a risk factor for ophthalmopathy in Graves' disease patients

Keywords: Serum Selenium Status, Egyptians Patients, Graves' Disease, Ophthalmopathy.

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Introduction

Thyrotrophic receptor antibody (TRAb) activation of the thyroid stimulating hormone (TSH) receptor results in an increase in thyroid hormone synthesis and release in Graves' disease (GD), an autoimmune thyroid condition.[1] Adult hyperthyroidism is mostly caused by GD, which is characterized by elevated levels of free thyroxine (FT4) and/or triiodothyronine (T3) and subnormal blood TSH levels, but it also involves nonthyroidal associated outcomes including pretibial myxedema, acropachy, and most commonly Graves' ophthalmopathy (GO) which affects 3-5% of GD patients.[2]

GO thyroid eye disease (TED) can cause the eyeball to protrude, the soft tissues around the eye to enlarge, and, less frequently, double vision. Up to 80% of people potentially have TED, yet only 10% of them exhibit symptoms that are clinically important, according to sensitive imaging investigations.[3] The pathological significance of TED within the orbit are over production of glycosaminoglycans (GAG), inflammation and adipogenesis. These processes are thought to be evoked at least partially by the release of local inflammatory cytokines.[4] Increased extraocular muscles and retrobulbar fat, which have been linked to inflammatory cytokines and excessive GAG release represent the main features of GO. These changes increase the volume of the orbital contents and may cause an increase in intra-orbital pressure.[5]

Reactive oxygen species (ROS) and free radicals are significantly more prevalent in individuals with GD due to an acceleration of their baseline metabolic condition.[6] The literature that is now accessible is mostly concerned with the protective benefits of powerful antioxidant systems as well as the involvement of oxidative stress in the development of GD. Superoxide dismutase, glutathione reductase, and glutathione peroxidase, among other intracellular antioxidant enzymes, are present in this system.[7]

In addition to being a vital trace element, selenium plays a significant role in boosting the generation of active thyroid hormone as well as acting as an antioxidant and anti-inflammatory.[8] Since the thyroid gland contains the highest levels of selenium. In selenoproteins such glutathione peroxidase, which catalyzes the breakdown of hydrogen peroxide and lipid hydroperoxide, the formation of which is enhanced in GD instances, selenium in the form of selenocysteine is integrated.[9] Selenium deficiency has a crucial impact on the onset and development of autoimmune thyroid illness because selenioproteins play a crucial role in thyroid autoimmune processes.[10]

Previous trials revealed a successful serial decrease in levels of serum thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody in Hashimoto's thyroiditis with selenium supplementation after 3–12 months.[11]

According to Dehina et al., 2016,[12] low selenium values are also linked to GO, and individuals with GO should be thought of as having a separate risk factor for relative selenium insufficiency.[13] Although the exact explanation of the improvement in GO is still unknown, one theory indicated that it is due to a decrease in oxidative stress since selenoproteins defend against ROS damage. Because baseline selenium levels in Graves' disease with ophthalmopathy compared to GD without ophthalmopathy have not been evaluated before, this study intended to test the hypothesis that selenium levels could be a risk factor for Graves' disease with ophthalmopathy.

Subjects and Methods

This was a case control study, carried out at the Endocrinology and Ophthalmology outpatient clinic, Ain Shams University Hospital, Cairo, Egypt during 2019-2021. Our study included 75 study subjects ≥ 18 years old. Study participants were categorized into 3 groups. Group I included 25 patients, diagnosed with GD but without GO. Group II included 25 patients, diagnosed with GD and with GO, divided into (mild, moderate, and severe GO). Group III included 25 apparently healthy subjects with no GD or GO as a control group.

Subjects excluded from the study included pregnant ladies and subjects with the following conditions bariatric surgery, chronic illness, gastrointestinal disorders or malabsorption syndrome, intravenous drug abusers, alcohol intake and smoking as these may affect selenium levels.

Clinical and Biochemical analyses

All patients were subjected to full history taken and clinical examination. They were examined for GO disease and disease severity by an ophthalmologist using the clinical activity score (CAS) classification of the American Thyroid Association**.** Laboratory investigations included thyroid function (free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH), TRAb, levels of TPOAb and

serum selenium. Venous blood samples (10 ml) were collected and centrifuged, then the serum was separated and kept at 80 °C until used.

Free triiodothyronine (FT3), free thyroxine (FT4), and thyroid-stimulating hormone (TSH) were estimated in serum samples using commercial enzyme linked immunoassay (ELISA) kits (BioVendor, North Carolina, USA), according to the manufacturer's instructions.

TRAB serum levels were evaluated by a third-generation ELISA kits (Thermo Scientific B.R.A.H.M.S, Hennigsdorf, Germany), according to the manufacturer's instructions. The TRAb kit reference range is 0- 1.75 IU/L. Serum TPOAb were assessed by an enhanced chemiluminescent microparticle immunoassay kits (COBAS Elecsys® kit, Roche Diagnostics, Switzerland), according to the manufacturer's instructions. The reference range of the anti-TPO kit is 0-35 IU/ml. Serum selenium levels were estimated for patients and controls using an atomic absorption spectroscopy (PerkinElmer, A Analyst 800 atomic absorption spectrophotometer, USA). A reference range for adults (60-150 ng/ml) was created using blood samples from 75 Egyptian blood donors.

Statistical Methods

Data were recorded on a computer and then analyzed using the Statistical Package for the Social Sciences (SPSS) software package version 20 (Armonk, NY: IBM Corporation).[14,(15] The verification of the normal distribution of quantitative variables was performed using the Kolmogorov-Smirnov test and the data were presented as mean and standard deviation as well as range (minimum and maximum). The Chi-square test was used for the comparison of qualitative variables between different groups. The ANOVA test was used for comparison between two studied groups regarding normally quantitative variables and Kruskal-Wallis H test used for comparison between the groups regarding abnormally quantitative variables. The statistical significance was determined at a *p*-value of <0.05.

Results

A total of 75 subjects were enrolled in the study, included 54 (72%) females and 21 (28%) males. There was no difference in the age of subjects with GO and GD. The average of disease duration was 2-6 years in Graves' orbitopathy and 2-7 years in Graves' disease. No presence of chronic illness or smoking habits were recorded among GO and GD. GO patients had mild, moderate, and severe disease.

Serum selenium was significantly different in patients with Graves' disease with orbitopathy compared with Graves' disease without orbitopathy. Serum selenium ranged between 30-55 and 528 ng/ml in Group I (patients with GD but without GO) and Group II (patients with GD and with GO), respectively ($p< 0.001$). Also, serum selenium level in patients with Graves' disease was significantly lower (30-55 ng/ml) than in control subjects (Group III, 67-140 ng/ml), $p < 0.001$ (Table 1, Figure 1). Furthermore, selenium levels were remarkably different in GO subgroups as it was 21-28, 18-22 and 5-16 ng/ml in the mild, moderate and sever GO disease, respectively ($p< 0.001$) (Table 2, Figure 2).

Table 1. Comparison of serum selenium between the study patient's groups.

Serum selenium	Group (I) (n=25)	Group (II) (n=25)	Group (III) (n=25)	*p* value
Min.-Max. ng/ml	30-55	5-28	140-67	<0.001
Mean± SD ng/ml	42.96±8.233	16.64±7.555	96.92±19.459	

Group I: patients with Graves' disease (GD) but without Graves' ophthalmopathy (GO)
Group II: patients with GD and with GO; Group III: control subjects.
*$P \leq 0.05$ is significant.

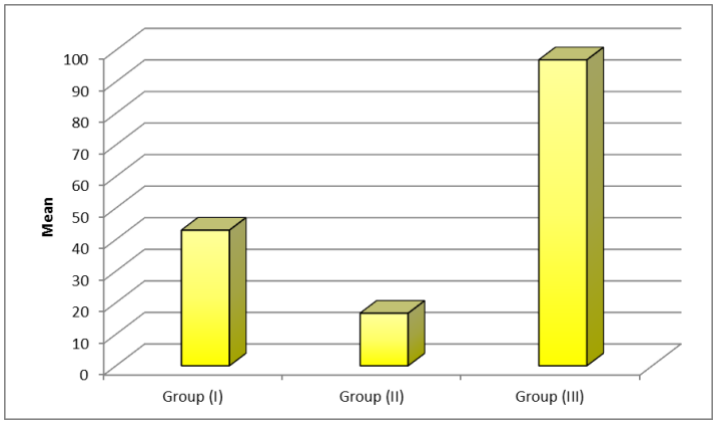

Figure 1. Comparison of serum selenium levels between patient's groups.

Table 2. Comparison of serum selenium between Graves' ophthalmopathy subgroups (Group II).

Serum selenium	Graves' Ophthalmopathy			*p* value
	Mild (n=8)	Moderate (n=5)	Severe (n=12)	
Min.-Max.	21-28	18-22	5-16	<0.001
Mean± SD	25.00±2.673	19.80±1.643	9.75±3.441	

*$P \leq 0.05$ is significant.

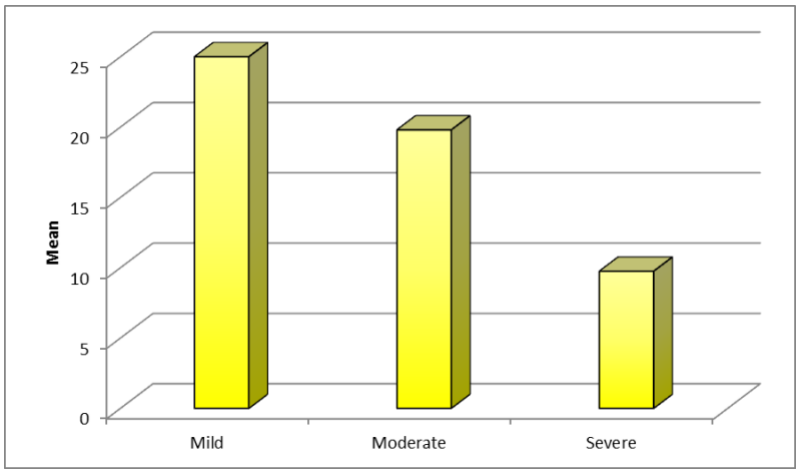

Figure 2. Comparison of serum selenium between Graves' ophthalmopathy subgroups (Group II).

Serum TRAb level was significantly different between the study groups, 4-28, 34-99 and 01.5 IU/L in Group I, II and III, respectively (p< 0.001). Also, serum TRAb level in Graves ophthalmopathy subgroups was significantly different (34-45, 52-62 and 58-98 IU/ L in mild, moderate and sever GO, respectively, p< 0.001) (Table 3).

Table 3. Comparison of serum TRAb between Graves' ophthalmopathy subgroups (Group II).

TRAb	Graves' Ophthalmopathy			*p* value
	Mild (n=8)	Moderate (n=5)	Severe (n=12)	
Min.-Max.	34-45	52-62	58-99	<0.001
Mean± SD	39.338±4.340	57.00±4.301	81.08±13.379	

*$P \leq 0.05$ is significant.

Serum TPOAb level was significantly lower in Group I (1.2-98 IU/ml) than in Group II (11-52 IU/ml) (p< 0.001) (Table 4). However, serum TPOAb level in the Graves' ophthalmopathy subgroups was not different (11-46, 32-37 and 12-52 IU/ml in the mild, moderate and sever GO, respectively) (p= 0.522).

Table 4. Comparisons of serum TPOAb level between the study groups.

TPOAb	Group (I) (n=25)	Group (II) (n=25)	Group (III) (n=25)	*p* value
Min.-Max.	1.2-98	11-52	0-29	<0.001
Mean± SD	34.54±33.523	32.16±9.970	12.92±8.869	

Group I: patients with Graves' disease (GD) but without Graves' ophthalmopathy (GO).
Group II: patients with GD and with GO; Group III: control subjects. *$P \leq 0.05$ is significant.

Correlations between selenium and different elements in groups I and II

Correlations between selenium and different elements in groups I and II are shown in Tables 5 and 6. There was a negative statistically significant correlation between selenium and each of TRAb (r= - 0.947, p <0.001) and TPOAb (r= - 0.856, p <0.001) in group I. Beside there was a negative potential relationship between selenium and TRAb (r= - 0.796, p<0.001) in group II. However, in group II there was no considerable correlation between selenium and TPOAb (r= - 0.228, p=0.272).

Correlations between selenium, FT3, FT4 and TSH were observed as follows. In group I, there was a remarkable negative correlation between selenium and each of FT3 (r= - 0.874, p<0.001) and FT4 (r= - 0.886, p<0.001). Furthermore, there was a significant positive association between selenium and TSH (r= 0.884, p<0.001) in group I. However, in group II there was no considerable association between selenium, FT3, FT4 and TSH (r= - 0.344, p=0.093, r= - 0.203, p=0.330 and r= 0.128, p=0.542, respectively).

Also, in group I we observed a remarkable positive association between TRAb and each of TPOAb (r= 0.820, p<0.001), Free T3 (r= 0.925, p<0.001) and Free T4 (r= 0.894, p<0.001). However, there was a remarkable negative relation between TRAb and TSH (r= - 0.826, p<0.001). With regard to group II, there was no

relationship between TRAb and each of TPOAb (r= 0.233, *p*=0.263) and FT4 (r= - 0.138, *p*=0.510). However, there was a significant positive correlation between TRAb and FT3 (r= 0.422, *p*=0.036).

In addition, in group I we observed a considerable positive relationship between TPOAb and each of TRAb (r= 0.820, *p*<0.001), Free T3 (r= 0.764, *p*<0.001) and Free T4 (r= 0.826, *p*<0.001) and a significant negative correlation between TPOAb and TSH (r= - 0.723, *p*<0.001). However, in group II there was no significant association between TPOAb and each of TRAb (r= 0.233, *p*=0.263), FT3 (r= - 0.052, *p*=0.806), FT4 (r= 0.095, *p*=0.651) and TSH (r= 0.164, *p*=0.432).

With regard to the clinical activity score (CAS) in group II, we observed a significant negative correlation between selenium and CAS (r= - 0.879, *p*<0.001). In addition, there was a significant positive correlation between TRAb and CAS (r= 0.878, *p*<0.001) in patients with Graves' orbitopathy. However, there was no significant correlation between TPOAb and CAS (r= 0.244, *p*=0.240).

Table 5. Correlation between serum levels of selenium, TRAb and TPOAb with different thyroid function tests in Group I (patients with Graves' disease but without Graves' ophthalmopathy).

Thyroid function tests	Selenium		TRAb		TPOAb	
	r	*p* value	r	*p* value	r	*p* value
TRAb	-0.947	<0.001			0.82	<0.001
TPOAb	-0.856	<0.001	0.82	<0.001		<0.001
TSH	0.884	<0.001	-0.826	<0.001	-0.723	<0.001
Free T3	-0.874	<0.001	0.925	<0.001	0.764	<0.001
Free T4	-0.886	<0.001	0.894	<0.001	0.826	<0.001

*$P \leq 0.05$ is significant.

Table 6. Correlation between serum levels of Selenium, TRAb and TPOAb with different thyroid function tests in Group II (patients with GD and with GO).

	Selenium		TRAb		TPOAb	
	r	*p* value	r	*p* value	r	*p* value
TRAb	-0.796	<0.001			0.233	NS
TPOAb	-0.228	NS	0.233	NS		
TSH	0.128	NS	-0.072	NS	0.164	NS
Free T3	-0.344	NS	0.422	0.036	-0.052	NS
Free T4	-0.203	NS	-0.138	NS	0.095	NS
Clinical activity score	-0.879	<0.001	0.878	<0.001	0.244	NS

$P > 0.05$ is not significant (NS).

Discussion

Selenium is an essential element, critical for the function of the thyroid, and particularly abundant in this gland.[16] Inflammatory processes of GD and GO are characterized by elevated oxidative stress with increased amounts of cytokines and free radical species.[6] GO could be improved by increasing selenium level by a variety of methods, including lowering oxidative stress in GO and by modifying immunological response and T-cell activities.[9]

Also, selenium may exert these positive benefits by lowering TPOAb and anti-thyroid-stimulating hormone receptor antibodies (TSHR-Ab) concentrations, regulating immunological systems, and suppressing orbital inflammation. Selenium appears to be helpful in reducing the inflammation of adipose tissue and extraocular muscle.[17]

Our study aimed to test the hypothesis that selenium levels could be a risk factor for GD with ophthalmopathy. Therefore, we assessed the level of serum selenium in patients with GD with and without ophthalmopathy. We also assessed thyroid hormones and antibodies. The study included 75 subjects. Of these, 50 patients were diagnosed with GD and 25 subjects as controls. Of the GD patients, 25 patients had GO as follows, 32% have mild GO, 20% have moderate GO and 48% have sever GO.

In our study we found a considerably lower serum selenium in Group II (subjects with orbitopathy) compared with Group I (subjects without orbitopathy). Serum selenium levels were significantly different as it was 30-55 ng/ml and 5-28 ng/ml in group I and II, respectively (p< 0.001). Beside we found a statistically significant variation in serum selenium level as it was lower in GD patients compared to control subjects (30-55 ng/ml and 67-140 ng/ml in group I and III, respectively p< 0.001). Additionally, selenium levels showed remarkable difference in GO subgroups as it was 21-28 ng/ml, 18-22 ng/ml, and 5-16 ng/ml in mild, moderate and sever GO, respectively (p< 0.001). So, it appears that the more progression of Graves' ophthalmopathy, the more reduction in selenium levels.

We also assessed thyroid function and antibodies in our study population. There was a significant variation in thyroid function and thyroid antibodies in patients suffering from GO compared with patients without orbitopathy. TRAb levels were significantly different as it was 4-28.5 IU/L and 34-99 IU/L in group I and II, respectively (p < 0.001). Also, TRAb levels were significantly different in GO subgroups, as it increased with more severity of GO (34-45 IU/ L, 52-62 IU/ L and 58-98 IU/ L in mild, moderate and sever GO, respectively, p< 0.001).

Regarding TPOAb level in groups I and II, there was a noticeable difference (1.298 IU/ml and 11-52 IU/ml in group I and II, respectively, p < 0.001). Also, there was no difference in its level in GO subgroups (11-46 IU/ml, 32-37 IU/ml and 12-52 IU/ml in mild, moderate and sever GO, respectively, p=0.522).

Regarding thyroid hormones (FT3 and FT4) there was a statistically significant difference as it was 3-11.5 pg/ml and 8-19.7 ng/dl in group I and II, respectively (p< 0.001). Regarding TSH level, there was a statistically significant difference as it was 0.1-2.8 µIU/mL and 0.01-0.2 µIU/mL in group I and II, respectively (p< 0.001). But in GO subgroups both FT3 and FT4 showed no difference as FT3 was 11-15.7 pg/ml, 8-13 pg/ml and 9.5-19.7 pg/ml and FT4 was 1.9-2.5 ng/dl, 1.8-2.4 ng/dl and 1.8-2.6 ng/dl in mild, moderate and sever GO, respectively (p< 0.028 and p=0.167, respectively). Also, TSH level showed no difference in GO subgroups as it was 0.1-2.8 µIU/mL and 0.01-0.05 µIU/mL, 0.04-0.2 µIU/mL, and 0.01-0.07 µIU/mL in mild, moderate and sever GO, respectively (p< 0.018).

To the best of our knowledge, the current study is unique, as we assessed selenium levels in individuals with Graves' orbitopathy compared to patients without Graves' orbitopathy in Egyptian subjects who consume enough selenium.

There have not been many studies on selenium supplementation for Graves' disease. In 2013, the protocol for the Graves' disease Selenium Supplementation trial (GRASS trial), a randomized controlled study to examine the impact of selenium supplementation in Graves' disease was published.[18] Selenium supplementation seems to be advantageous in

thyroid hormone production, although it only has an advantageous effect when nutritional intake is insufficient. People who already consume enough selenium could develop type 2 diabetes if they take extra selenium supplements.[19]

The thyroid glandular tissue contains the highest concentration of selenium in the human body. The study by Dickson and Tomlinson, 1967, demonstrated the significance of selenium in thyroid physiology. Therefore, selenium supplementation has a positive impact on mild inflammatory ophthalmopathy in people with Graves' disease and speeds up the process of achieving euthyroidism.[19]

A study by Marcocci et al., 2011, found that selenium has considerably enhanced soft tissue alterations, aperture of eyelid, and manifestation in mild thyroid orbitopathy.[7] It also greatly decreased advancement of mild active thyroid orbitopathy and slowed its progression. The benefits of selenium supplementation in GO were proven in a randomized control trial, included 150 patients with GO who received 100 grammes of selenium twice daily for six months as opposed to a placebo or pentoxifylline 600 mg twice a day. When compared to placebo at the 6-month evaluation, selenium treatment, but not with pentoxifylline, was linked with a better life quality (p=0.001), less involvement of eye (p=0.01), and a slowing of the course of Graves' orbitopathy (p=0.01).

Further randomized controlled studies on the treatment using selenium supplements for Graves' orbitopathy are necessary to confirm the advantages in people with varying baseline selenium statuses and to establish the ideal formulation and dosage of selenium. In a European population where they were known to be a slight selenium shortage, supplement of selenium over six months in Graves' orbitopathy was not linked to any negative effects.[7]

As revealed by Pedersen et al., 2013[20] study in a Danish population, patients recently diagnosed with Graves' disease had lower serum selenium levels than randomly chosen normal controls (mean 114 µM vs 125 µM, p<0. 001), and patients with autoimmune hypothyroidism had slightly lower serum selenium levels than controls. The level of selenium was not related to the patients' thyroid function status. They concluded that autoimmune thyroid disease and selenium insufficiency are related.

In the same line, the study by Wertenbruch et al., 2007,[21] compared serum level of selenium in 83 cases with Graves' disease in relapse or remission. They found no difference between the two groups (mean 0.92± 0.28 µM, vs 0.91± 0.21µM), though they did find the highest levels of selenium (>152 µM) in patients suffering from Graves' disease in remission.

According to a study by Khong et al., 2014,[13] there was a marginally significant drop in selenium levels in cases with GO compared to people without the condition. They observed that 76% of the participants which included 83 Graves' orbitopathy cases and 72 Graves' disease controls have serum selenium levels below the optimal range of 100 lg/l (1.27 µM), which correlates with glutathione peroxidase activity, 1% of the GO and GD subjects in this study population, respectively, fell into the category of having serum selenium levels < 0.75 µM, which is seemed inadequate. The lowest bounds of the 95% confidence interval for both the two GD controls (2%), as well as the nine GO cases (9%), were 0.83 µM. As a result, this study population had a generally low selenium status. This may indicate that, despite the fact that the selenium content in GO was only slightly lower than its level in GD, the orbit's ability to handle higher oxidative stress was hampered because selenium level was less than the ideal status.

The study by Khong et al., 2014, also indicated that the small mean selenium difference between GO and GD remains uncertain, as level of selenium in plasma does not represent selenium level in tissue and the absolute measurement may underestimate local selenium requirements.[13] This is because the physiology and importance of the selenium variation between Graves' orbitopathy and Graves' disease is still unknown. In addition to assessing thyroid hormones and antibodies, which appear to have a strong relationship with selenium, our study offered the significant benefit of proving that serum level of selenium was considerably lower in GO than GD. Finally,

we may conclude that serum selenium levels were lower in GO patients compared with GD patients in an Egyptian population. Low selenium levels may be a risk factor for ophthalmopathy in Graves' disease patients

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Author Contributions

All authors shared the design of this study. MSM, MMM, AMA, HMA, HKM and GAH proposed the idea. Data collection and sampling was done by GAH. Data analysis and interpretation were done by GAH, HKM, MMM and MSM. Writing and revision of the manuscript were done by MRH, MMM, AMA, HMA, HKM and GAH. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The Research Ethical Committee of the Faculty of Medicine, Ain Shams University reviewed and approved the study protocol (MD 320 /2019).

Informed consent

Each participant provided a written informed consent before being included in the study.

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