The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89 (2), Page 6762- 6766

Study of Selenium Status in Grave's Disease Patients

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ABSTRACT

Background: Grave's disease (GD) is an autoimmune disorder caused by the thyroid receptor antibodies (TRAb), resulting in hyperthyroidism and goiter with extrathyroidal manifestations as; Grave's ophthalmopathy, dermopathy and acropachy. Selenium: the essential trace element found to have a crucial role in the maintenance of thyroid physiology and function. Thus, selenium deficiency reported to be linked in the start and progress of autoimmune thyroid diseases in genetically predisposed individuals. The aim of the current to evaluate the selenium status by measuring serum selenoprotein P (SEPP) level in GD patients, in comparison to healthy subjects and assess the linkage between selenium status and Grave's ophthalmopathy (GO).

Patients and methods: A case control study that was conducted on 80 subjects; Group (A): 40 patients with GD and Group (B): 40 healthy control subjects recruited from endocrinology clinic at Ain Shams University Hospitals in the period between December 2020 and June 2021. All the included GD patients suffered from GO.

Results: SEPP levels "as a marker of selenium status" were significantly lower in GD patients than control subjects. No significant correlation was found between selenium status and GO severity or activity.

Conclusion: GD patients are markedly selenium deficient. There is lack of association between selenium status and GO severity and activity.

Keywords: Grave's disease, Ophthalmopathy, Selenium, Selenoprotein P.

INTRODUCTION

Grave's disease (GD); the autoimmune disorder that affects mainly the thyroid gland with extrathyroidal manifestations like Grave's ophthalmopathy dermopathy and acropachy in less common cases, is the most common cause of hyperthyroidism in iodine-sufficient countries [(1)]. It is characterized by the presence of circulating autoantibodies (TRAb), resulting in hyperthyroidism and goiter. GD has multi-factorial etiology; affected by gender, age, iodine and selenium supply, smoking, infections, stress and other auto-immune diseases [(2)].

Selenium; the essential element that has several biological effects depending on the level of its intake, is integrated into 25 selenoproteins, important for endocrinal functions, immunity, metabolism, and cellular homeostasis. Selenium has a vital role in the metabolism of thyroid hormones [(3)]. Selenium deficiency was found to be associated with many thyroid diseases especially that of autoimmune pathogenesis, cell-mediated and the humoral immune response may be impaired in selenium deficiency [(4)].

Selenium status is of increasing concern to define the risk of nutritional deficiency of selenium, to estimate its role in decreasing cancer risk, assess public health and to consider the pros and cons of excess selenium [(5)]. It is essential to study selenium status in different countries of the world, due to the variability of selenium status and the variability of its correlation with thyroid diseases [(6)].

Selenoprotein P represents 50% of selenium in blood [(7)]. Accordingly, serum selenium is mirrored by circulating selenoprotein P concentrations especially in marginally supplied individuals [(8)].

The current study aims to evaluate the selenium status by measuring serum selenoprotein P (SEPP) level in GD patients, in comparison to healthy subjects and assess the linkage between selenium status and Grave's ophthalmopathy (GO).

PATIENTS AND METHODS

This is a case control study that was conducted on 80 subjects; Group (A): 40 patients diagnosed with GD and Group (B): 40 healthy control subjects recruited from Endocrinology clinic at Ain Shams university hospitals in the period between December 2020 and June 2021. All the included GD patients suffered from GO.

Inclusion criteria:

1. Age: from 18 to 55 years old, both sexes will be included.
2. Confirmed Grave's patients with GO

Exclusion criteria:

1. Patients less than 18 years old and more than 55 years old.
2. Subjects on selenium supplementation.
3. Pregnant and lactating females.
4. Major organ failure.
5. Psychiatric illness.

- **The following data was recorded and analyzed:**

Group (1) "GD patients" were subjected to:

1. History taking and clinical examination.
2. Confirmation of Grave's disease diagnosis clinically and laboratory thyrotoxic lab profile and evidence of GO.

Received: 12/7/2022
Accepted: 15/9/2022

3. Laboratory data: Level of blood selenoprotein P, and level of TRAb antibodies.
4. Assessment of GO activity according to CAS and severity according to EUGOGO classification.

Group (2) "control subjects" were subjected to:

1. History taking and clinical examination.
5. Laboratory data: Levels of TSH, free T3, free T4, to confirm euthyroidism. Level of blood selenoprotein P, and Level of TRAb antibodies.

- **Data collection and sampling:**

Selenoprotein P measurement:

Blood samples were collected in tubes without using anticoagulants and allowed to clot for 10 -20 minutes at room temperature, centrifuged at 2000-3000 RPM for 20 minutes then stored at 2-8°C. SEPP levels were measured using an Enzyme-Linked Immunosorbent Assay (ELISA) kit (Wuhan, China) with coefficient of variation < 10%.

TSH, FT3, FT4 and Thyroid-stimulating Hormone Receptor Antibody (TRAb) measurement:

Blood samples were collected in tubes without using anticoagulants, centrifuged then stored at -20 C until analysis. TSH, FT3 and FT4 were measured in the same day using chemiluminescence assay (USA). TRAB was measured using an Enzyme-Linked Immunosorbent Assay (ELISA) kit (China).

Ethical consent:

This study was ethically approved by the Institutional Review Board of the Faculty of Medicine, Ain Shams University, Written informed consent was taken from all participants. The study was conducted according to the Declaration of Helsinki.

Statistical Analysis

Recorded data were coded, reviewed and analyzed using the SPSS version 25.0 (Armonk, NY: IBM Corp). Normality test was done on the data using Kolmogorov-Smirnov test to detect normal distribution of the data. Quantitative data were expressed as mean and standard deviation (SD) or median and range. Qualitative data were expressed as frequency and percentage.

The comparison between groups regarding qualitative data was done by using Chi square ($\chi 2$): Used to test the association between two categorical variables or to detect difference between two or more proportions, Student's t test (t): Used to test the significant difference between two normally distributed independent variables or to detect difference between two means, Mann Whitney test (U): Used to test the significant difference between two non-normally distributed independent variables, Pearson Correlation (r): Used to measure the strength of a linear association between two continuous variables, Receiver Operating Characteristics (ROC curve): Used to measure the performance of ability of test to discriminate whether the condition is present or not and to assess the best cut off point with its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under curve (AUC). P value $\leq$0.05 was considered significant.

RESULTS

GD patients had significantly lower levels of selenoprotein levels than control group (Table 1).

Table (1) shows comparative analysis of laboratory data of group (A) "GD patients" and group (B) "control subjects":

Variable	Group (A) (GD)		Group (B) (control)		Test of significance (P value)
	Mean	SD	Mean	SD	
TSH (uIU/ml)	**0.08**	**0.01**	**1.66**	**0.31**	**(U= 1.581, p<0.001*)**
Free T4 (ng/dl)	**2.7**	**0.5**	**1.6**	**0.30**	**(U= 452, p= 0.001*)**
Free T3 (pg/ml)	**6**	**1.3**	**2.8**	**0.42**	**(U= 265, p<0.001*)**
TRAb (IU/ml)	**87.2**	**21.7**	**12.4**	**2.91**	**(U= 11, p<0.001*)**
Selenoprotein P (ng/ml)	**15.37**	**3.4**	**80.7**	**18.4**	**(U= 1.417, p<0.001*)**

U; Mann-Whitney test. *; Significant

Smokers had significantly lower levels of SEPP than non-smokers GD patients (Table 2).

Table (2): Comparison between smokers and non-smokers among GD patiens as rgard SEPP levels

Selenoprotein P (ng/ml)	Smoking		Test of significance
	Smokers (n= 9)	Non-smokers (n= 31)	
Mean ± SD	12.06 ± 2.87	16.34 ± 4.01	(U= 77.5, p= 0.044*)

U; Mann Whitney test. * Significant (p<0.05)

This table shows higher SEPP levels in females, but this difference is not statistically significant (U= 84.5, p= 0.14) (**Table 3**).

Table (3): Comparison between males and females among GD patiens as regard SEPP levels.

Selenoprotein P (ng/ml)	**Sex**		**Test of significance**
	Male (n= 8)	**Female (n= 32)**	
Mean ± SD	13.38 ± 3.11	15.88 ± 3.84	(U= 84.5, p= 0.14)

U; Mann Whitney test. * Significant (p<0.05)

No statistically significant correlation between selenoprotein P and age and duration (**Table 4**).

Table (4) shows correlation between Selenoprotein P level and age and duration of GD in group (A) "GD patients":

Variable	**Selenoprotein P (ng/ml)**	
	r	**P**
Age (years)	-0.145	0.37
Duration of Grave's disease (years)	0.116	0.478

Higher selenoprotein P levels found in active and mild GO patients but with non-significant difference (**Table 5**).

Table (5) shows Selenoprotein P level (mean ± SD) in relation to GO activity and severity in group (A) "GD patients".

GO disease status	**Selenoprotein P (mg/l)**
Mild	15.95 ± 3.9
Moderate	14.8 ± 3.02
Active	17.19 ± 4.16
Inactive	12.66 ± 2.93

Selenoprotein levels showed significant positive correlation with FT4 and weak negative non-significant correlation with TRAb levels (**Table 6**).

Table (6) shows correlation of Selenoprotein P with laboratory tests in group (A) "GD patients":

Variable		**Free T3 (ng/dl)**	**Free T4 (ng/ml)**	**TSH (uIU/ml)**	**TRAb (IU/ml)**	**T3/T4 Ratio**
Selenoprotein P (ng/ml)	**R**	0.112	0.424	0.186	-0.146	-0.283
	P	0.49	**0.006***	0.25	0.37	0.077

r; Pearson Correlation. * Significant (p<0.05)

Table 7 and Figure 1 showed a statistically significant diagnostic ability of selenoprotein P in detection of Grave's disease as with 100% sensitivity, 62.5% specificity and a cut off value of ≤37.

Table (7): ROC curve analysis between group (A) "GD patients" and group (B) "control subjects" as regards selenoprotein P level:

AUC	**95% CI**	**Cut off point**	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**+PV (95% CI)**	**-PV (95% CI)**	**P-value**
0.886	0.795 – 0.946	≤37	100% (91.2% - 100%)	62.5% (45.8% - 77.3%)	72.7% (64.1% - 79.9%)	100%	<0.001*

AUC: Area under the curve. +PV: positive predictive value. -PV: negative predictive value

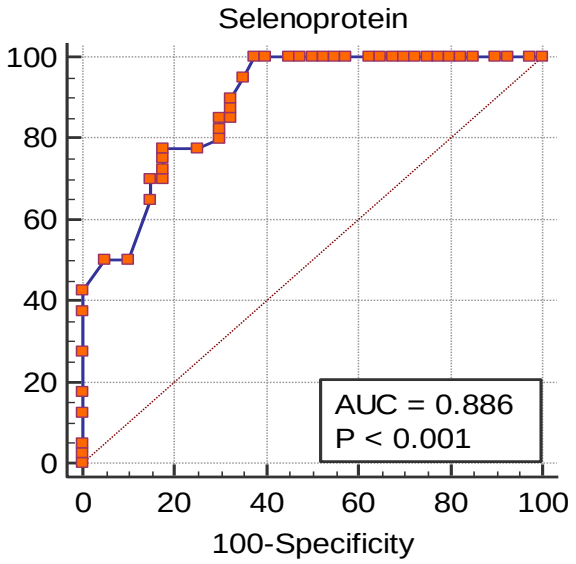

Figure (1): ROC curve analysis between group (A) "GD patients" and group (B) "control subjects" as regards selenoprotein P level.

DISCUSSION

GD, the commonest cause of thyrotoxicosis, is an auto-immune disorder that causes diffuse goiter, hyperthyroidism, ophthalmopathy and dermopathy [(9)]. In GD, T lymphocytes are sensitized to antigens in the thyroid gland and enhance B lymphocytes to form antibodies, especially TRAb. GD has multifactorial etiology including heredity, gender, age and stress [(10)].

Selenium is important for human wellbeing, it is vital for maintenance of immunity, endocrine function, metabolism and cellular homeostasis. The thyroid gland has a maximum level of selenium between other organs. Selenoproteins act as antioxidants, they are also essential for metabolism of thyroid hormones [(3)]. Mean serum selenium levels vary according to age, sex, race, ethnicity, and geographic region [(11)]. Thus, Selenium status needs to be evaluated only in comparison to precisely matched controls.

This study aims to evaluate the selenium status by measuring serum SEPP level in GD patients in comparison to healthy subjects and assess the linkage between selenium status and GO.

This study included 2 groups; Group (A) 40 patients presented with GD and Group (B) 40 healthy control subjects attended endocrinology clinic in Ain Shams University Hospitals.

In group (A), there was female predominance in GD patients, this can be attributed to higher risk of developing autoimmune thyroid disease in females [(12)]. All included subjects in group (**A**) suffered from GO; 40% had inactive GO while 60% had active GO, according to CAS. 50% had mild disease and the rest had moderate GO, according to EUGOGO classification.

There was highly significant difference between group (**A**; GD patients) and group (B; control subjects) ($p< 0.001$*), as regard thyroid panel; Free T3, Free T4, TRAb levels were significantly higher, while TSH was significantly lower in group (A; GD patients) as illustrated in **table 1**. In addition, mean SEPP level in (A; GD patients) was significantly lower than in group (B;control subjects) (15.37 VS 80.7, $P<0.001$), this agrees with **Federige *et al.*** [(13)], who showed significantly lower SEPP level in GO patients than matched controls. However, no significant difference was found between GD patients without ophthalmopathy and control subjects. On the other hand, **Krassas *et al.*** [(14)] found no significant difference between selenoprotein P level in GD patients compared to controls, and they explained it by small number of studied control subjects. However, selenoprotein P level in GD patients was lower than non-autoimmune thyroid diseases' patients, which would represent inflammatory reactions with a consequent increase in consumption of selenium dependent proteins in attempt to prevent the production of free radicles generated by thyroid autoimmune aggression [(14)].

Regarding the relation between SEPP level and age, sex and smoking, our study showed non-significant weak negative correlation with age, this could partially agree to the inverse correlation between age and SEPP reported by **Duntas & Benvenga** [(15)]. In contrary, the study of **Krassas *et al.*** [(14)] found positive correlation between age and selenoprotein P. On the other hand there was no significant difference in SEPP in both sexes. This disagrees with **Krassas *et al.*** [(14)] **and Duntas & Benvenga** [(15)] who mentioned higher levels of selenoprotein P in males. This may be due to small number of male subjects in our study. Smokers had significantly lower level of SEPP in our study. This goes in agreement with **Duntas & Benvenga** [(15)], while a study by **Dehina *et al.*** [(16)] showed no difference in selenoprotein P in smokers compared to nonsmokers. In fact smoking found to be associated with decreased levels of antioxidants but the mechanism is not known exactly.

SEPP and duration of GD had weak positive not statistically significant correlation in our study, this goes in agreement with **Krassas *et al.*** [(14)] who attributed the lower selenoprotein P level in newly diagnosed GD patients than old one, to the effect of carimazole which may have reduced inflammatory process and cellular immunity, thereby further increasing Selenoprotein P levels.

As regard the relation between SEPP levels and different grades of GO severity and activity, our study revealed higher levels of SEPP levels in mild GO versus moderate cases, which agreed with **Dehina *et al.*** [(16)] and would represent the importance of selenium supplementation in GO patients to decrease risk of severity as illustrated in **table 5**. However, in contrast to our expectations, our study revealed higher SEPP levels in active GO versus inactive cases. Although it is known that inflammatory stimuli reduce SEPP biosynthesis

causing low levels of it, but we can find an explanation for this result which lies in the local nature of the inflammatory process of GO having little effect on systemic proinflamatory cytokines concentrations and thus not directly affect SEPP biosynthesis in liver which affects systemic selenium status [17].

The relation between selenium status and thyroid function still controversial; as our study revealed non-significant correlation between SEPP levels and TSH and free T3 while FT4 revealed positive significant correlation with the decreased levels of SEPP. FT3/FT4 ratio also showed negative low significant correlation with SEPP with p value 0.07, TRAB also showed weak negative non-statistically significant correlation with SEPP level in our study. **Dehina *et al.*** [16] reported non-significant correlation between SEPP levels and thyroid function nor TRAB. While, **Franczek-Jucha *et al.*** [18] reported weak correlation between serum selenium and FT3and FT4 levels as well. In fact it was found that selenium deficiency affects the function of iodothyronine deiodinase (DIOS), which are selenoproteins responsible for T4 to T3 conversion, which explains why selenium deficiency causes high FT4 and low FT3/FT4 ratio levels [19].

Finally, our study interpreted a statistically significant diagnostic ability of SEPP in the detection of GD as with 100% sensitivity, 62.5% specificity and a cut off value of ≤37, as illustrated in table (7). But, more studies needed to support this finding and apply it in practice with evaluation of the effect of selenium supplementation on disease remission.

In conclusion, our study interpreted that selenoprotein P "as a marker of selenium status" is significantly lower in GD patients. All GO patients are markedly selenium deficient. There is lack of association between selenium status and GO activity and severity.

Financial support and sponsorship: Nil.
Conflict of interest: Nil.

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