



# Involvement of the Selenium Level in Plasma Glutathione Peroxidase Activity in Newly Diagnosed Patients with Graves' Disease and Hashimoto Thyroiditis

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## ABSTRACT

Disturbed antioxidant enzymes activities was known to be associated to Auto-Immune Thyroid Diseases (AITD), but it is noteworthy that the selenoenzyme glutathione peroxidase (GPx) showed the lowest activity and a selenium (Se) deficiency may be the major cause. Most studies conducted on AITD are focused on the effect of the Se supplementation on the evolution of these pathologies. The aim of this study was to evaluate the Se level in plasma of Graves' Disease (GD) and Hashimoto Thyroiditis (HT) patients and its relationship with the redox status of these AITD. For this purpose, the Se levels as well as the GPx activity were evaluated in the plasmas of 33 patients with AITD. Results were compared with those of 27 healthy controls and among the two groups of patients. Results concerning GD and HT patients revealed lower Se levels by comparison with healthy controls ( $p < 0.01$  and  $p < 0.05$  respectively). Just like the Se, the GPx activity was found to be significantly lower in GD and HT patients compared to controls ( $p < 0.01$ ,  $p < 0.001$  respectively). The comparison between AITD groups showed higher Se level for the HT group ( $p < 0.05$ ), whereas, no significant differences were noted for the GPx activity. The correlation study showed positive correlation between Se level and GPx activity in HT group ( $r = 0.64$ ,  $p < 0.01$ ); and negative correlations between Se and anti-thyroglobuline and anti-Thyroid Stimulating Hormone Receptor levels in GD group ( $r = -0.71$ ,  $p < 0.05$  and  $r = -0.73$ ,  $p < 0.05$  respectively). As a conclusion, our findings have shown that the Se deficiency may be involved in the redox misbalance in HT; further investigations are mandatory to elucidate the origin of the reduced GPx activity in GD patients.

## Indexing terms/Keywords

Selenium; Glutathion peroxidase; autoantibody; Graves' disease; Hashimoto Thyroiditis.

## Academic Discipline And Sub-Disciplines

Biomedical Sciences

## SUBJECT CLASSIFICATION

Autoimmune Diseases; Endocrine Pathology

## TYPE (METHOD/APPROACH)

Research Article

## INTRODUCTION

The trace element selenium (Se) is a nonmetal mineral that is attained through consumption of a wide variety of dietary components [1]. This essential micronutrient occurs in the form of the amino acid selenocysteine in selenoproteins which are required for the correct functioning of the immune system with a recommended daily intake for adults of 55  $\mu\text{g}$  [2, 3]. Besides, selenoproteins presented effects on redox homeostasis, reproduction, thyroid hormone metabolism and redox processes in thyroid cells [4]. Recently, it has been established that Se is capable of modifying the expression of the selenoenzyme glutathione peroxidases (GPx) which is involved in the regulation of the redox state and the protection from oxidative damage in thyrocytes [2,5,6]. The GPx enzyme play a crucial role in the thyroid gland by eliminating the hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) produced in thyrocytes during the hormonogenesis [5]. Nevertheless, few studies were interested in the Se level in Auto-Immune Thyroid Diseases (AITD) and its involvement in the disturbed oxidative profile. Recent work revealed that the Se deficiency is associated to disturbed oxidative profile in plasma of Hashitoxicosis patients' [7]. The majority of studies conducted on AITD are focused on the effect of the Se supplementation on the evolution of these pathologies. Several investigations showed that the Se supplementation can enhance the biochemical restoration of hyperthyroidism in Graves' thyrotoxicosis and reduce the anti-thyroperoxidase (anti-TPO) level in autoimmune thyroiditis [8, 9, 10]. The aim of this study is to evaluate the Se level in the plasma of newly diagnosed patients with Graves' Disease (GD) and Hashimoto Thyroiditis (HT). After that, and in order to investigate the involvement of the Se in the redox status and the AITD establishment, correlation study was undertaken between the Se level and the GPx activity in one hand the anti-thyroid autoantibodies and the thyroid hormones and in the other.

## PATIENTS AND METHODS

### Patients

The study was approved by the local ethics committee of the Hedi Chaker University Hospital of Sfax, Tunisia. Study subjects were recruited from the department of endocrinology inpatient and outpatient clinics, Hedi Chaker, University Hospital of Sfax, Tunisia. The study group included 33 plasmas of untreated Tunisian patients suffering from HT and GD. The control group comprised 27 healthy volunteers who had no family history of AITD [Table 1]. The identification of GD was based on the presence of biochemical hyperthyroidism, as indicated by a decrease in the thyroid-stimulating hormone (TSH), an increase in the free thyroxine (FT4), the free tri-iodothyronine (FT3) and the anti- Thyroid Stimulating Hormone Receptor (TRAb) antibody levels in association with diffuse goiter or the presence of exophthalmos [Table 2]. The diagnosis of HT was based on the presence of thyroid hormone-replaced primary hypothyroidism defined as a TSH level above the upper limits associated with positive titers of thyroid auto-antibodies (anti-TPO or anti-Tg) and a palpable goiter [Table 2]. Venous blood samples from patients and controls were centrifuged at 1500×g for 10 min and plasma was separated and stored in small aliquots. All samples of plasma were immediately frozen and stored at -80°C until being analyzed.

### Laboratory Studies

The TSH, FT4 and FT3 levels were determined by commercial kits (RIA-gnost® FT4, RIA-gnost® FT3 and Vidas TSH, bio merieux, France). The anti-thyroid autoantibodies, TRAb, anti-TPO and anti-Tg antibody levels were determined by enzyme-linked immunosorbent assay (Orgentec, Germany). Selenium plasma concentration was determined by atomic absorption spectrophotometry [11]. Antioxidant GPx activity was performed according to the spectrophotometric method of Flohe and Gunzler (1984) which is based on glutathione oxidation by GPx in the presence of the 5,5'-dithiobis 2-nitrobenzoate (DTNB). The absorbance was determined at 412 nm. GPx activity was expressed as  $\mu\text{M}$  of disappeared reduced glutathione (GSH)/min/mg proteins [12]. Protein Assay Kit from Bio-Rad (France) was used to determine the protein level in the plasmas of patients and controls. The bovine serum albumin served as a standard.

### Statistical Analyses

The one-way ANOVA variance analysis was used to assess the statistical significance of the differences between patients and controls in order to evaluate the Se level and the GPx activity. When a significant F ratio was found, the Tukey multi-comparison test was used to determine the statistical significance between the means. The correlation study was assessed using the Pearson correlation test. All data are expressed as means  $\pm$  standard error mean (S.E.M). The level of significance was taken as  $p < 0.05$ . The statistical analyses and the figures were performed using GraphPad Prism 6.

## RESULTS

### Selenium Level in Plasmas of AITD

The Se level was found to be lower in patients with GD and HT compared to controls ( $p < 0.01$  and  $p < 0.05$  respectively). When AITD were compared, Se level was higher in patients with HT than in patients with GD ( $p < 0.05$ ) Fig 1.

### Glutathione Peroxidase Activity in Plasmas of AITD

The GPx activity was found to be lower in plasma of GD and HT patients with comparison to controls ( $p < 0.01$  and  $p < 0.001$  respectively). However, no statistical differences were observed when both AITD were compared Fig 2.

### Correlation Study

Significant positive correlation was obtained between the Se level and the GPx activity only in HT patients ( $r = 0.64$ ,  $p < 0.01$ ) Fig 3. In addition, correlation study between the Se and the thyroid autoantibodies levels showed negative correlations between the Se and the anti-Tg ( $r = -0.71$ ,  $p < 0.05$ , Fig 4) as well as the TRAb levels in GD patients ( $r = -0.73$ ,  $p < 0.05$ , Fig 5). No correlations were found between the Se and the thyroid hormones levels in both AITD.

## DISCUSSION

This study was focused on the Se level in AITD and its involvement in the redox status imbalance as well as in the evolution and/or the development of these thyroid pathologies.

Our results demonstrate lower Se level in plasmas of newly diagnosed patients with GD and HT compared to healthy controls. These findings are in accordance with several studies showing that the Se status may have an impact on the thyroid pathologies development. Indeed, low Se level was associated with Hashitoxicosis, Graves' ophthalmopathy, goiter [7, 13, 15, 16]. In regard to the GD patients, our study seems to be the first investigation revealing a negative correlation between the Se and the anti-Tg as well as the TRAb antibodies levels. This data indicated that the Se level may be involved in the high level of the anti-thyroid antibodies in GD cases and so an effect on thyroidal autoimmune process. Our results are in accordance with the study by Wertenbruch and colleagues (2007) compared serum Se levels in patients with remission and relapse of GD. The authors found highest serum Se level in the remission group which indicates a positive effect of the Se on the outcome of GD [17]. Furthermore, it was observed that Se decreases the formation of proinflammatory cytokines and contributes in synergy with the anti-thyroid drugs to the stabilization of the autoimmune process in GD and slowed the progression of Graves' ophthalmopathy [18, 19].

The Se status is closely related to the physiological and pathological effects of selenoproteins. Indeed, selenoproteins such as GPx, thioredoxin reductases, and iodothyronine deiodinases are involved in redox reactions, and the selenocysteine is an active-site residue essential for the catalytic activity [20, 21]. The GPx is expressed in the thyroid gland in large quantities and its main function is to protect the body against damage caused by Reactive Oxygen Species (ROS) [22]. Our results concerning both AITD patients revealed a significant decrease in the plasmatic GPx level with comparison to healthy controls. These results confirmed previous studies showing reduced GPx level in HT, GD, Hashitoxicosis and papillary thyroid carcinoma patients in comparison to controls [7, 23]. Moreover, our results concerning HT patients showed a significant and positive correlation between the Se level and the plasmatic GPx activity. These results enable us to hypothesize that Se deficiency may be one of the main causes of the low GPx activity found in HT patients. Furthermore, these findings support the hypothesis that the Se level may have an impact on the antioxidant activity in the thyroid pathologies [22]. The selenoenzyme GPx, is involved in the protection of the thyroid from the H<sub>2</sub>O<sub>2</sub> that represents a crucial co-factor for the TPO enzyme catalyzing the iodination and the coupling of tyrosyl residues in the Tg to produce the thyroid hormones [2, 16]. GPx-3 is the only extracellular member of the GPx family; it represents therefore all the GPx activity in the plasma and may protect cells against ROS in the extracellular environment [20, 24]. The study undertaken by Barrett *et al.* (2013) suggested that the loss of the GPx-3 would enhance the tumorigenesis in inflammatory carcinogenesis which is a rich process with ROS production [25]. In contrast to HT pathology, GD one did not show correlation between the Se deficiency and the low GPx activity. Among hypothesis, the promoter hypermethylation seems to be the source of the decreased expression of GPx-3 described in the thyroid [24, 25]. Even so, other factors that may cause reduced GPx activity should not be excluded, such as the low level of the glutathione reductase [26]. In fact, GPx protect cells from oxidative damage by catalyzing the conversion of H<sub>2</sub>O<sub>2</sub> into water and oxygen through the oxidization of the glutathione. The oxidized glutathione will be converted into its reduced form by the glutathione reductase [27]. Further investigations are mandatory to elucidate which pathways are incriminated in the reduction of the GPx activity in GD.

## CONCLUSION

In conclusion, the results of this study indicate that the Se deficiency may be involved in the misbalance of the redox status in HT and the high level of the anti-thyroid autoantibodies in GD cases. Nevertheless, further studies are mandatory for confirmation of this study's findings and justification of the Se supplementation in both AITD.

**Table 1. Basic clinical characteristics of patients and healthy controls. Statistical analyses were performed using one-way ANOVA test, there were no significant differences between the groups regarding the age ( $p > 0.05$ )**

Parameters	Controls	HT	GD
Number	27	21	12
Age (years)	42.4±10	49±10.7	40.6±16.2
Gender (Female/Male)	18/9	16/5	7/5

**Table 2. The thyroid hormones and the anti-thyroid autoantibodies levels in the plasma of GD, HT patients and controls. The results are expressed as mean±S.E.M. Statistical analyses were performed using one-way ANOVA test, significant differences were found \*\*\*  $p < 0.001$**

Parameters	Controls	HT	GD
FT4 (pg/ml)	11.07±1.22	2.38±0.43***	57.1±5.23***
FT3 (pg/ml)	2.95±0.1	1.24±0.12***	12.69±1.57***
TSH (μUI/ml)	1.5±0.12	9.81±1.28***	0.08±0.01***
Anti-TPO (UI/ml)	<70	760±203	481±156
Anti-Tg (UI/ml)	<100	742±307	436±291
Anti-R-TSH (μUI/ml)	<2	--	37±2.9

## FIGURES

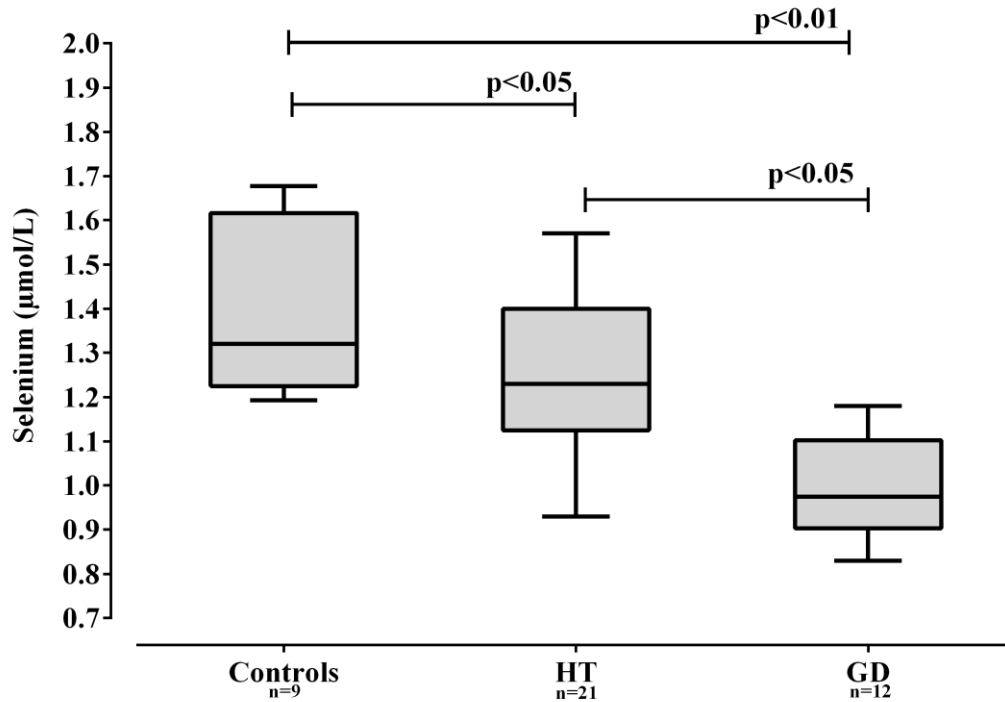


Fig 1: The glutathione peroxidase levels in the plasma of GD, HT patients and controls. The results are expressed as mean±S.E.M. Statistical analyses were performed Tukey multi-comparison test, significant differences were found for p<0.05.

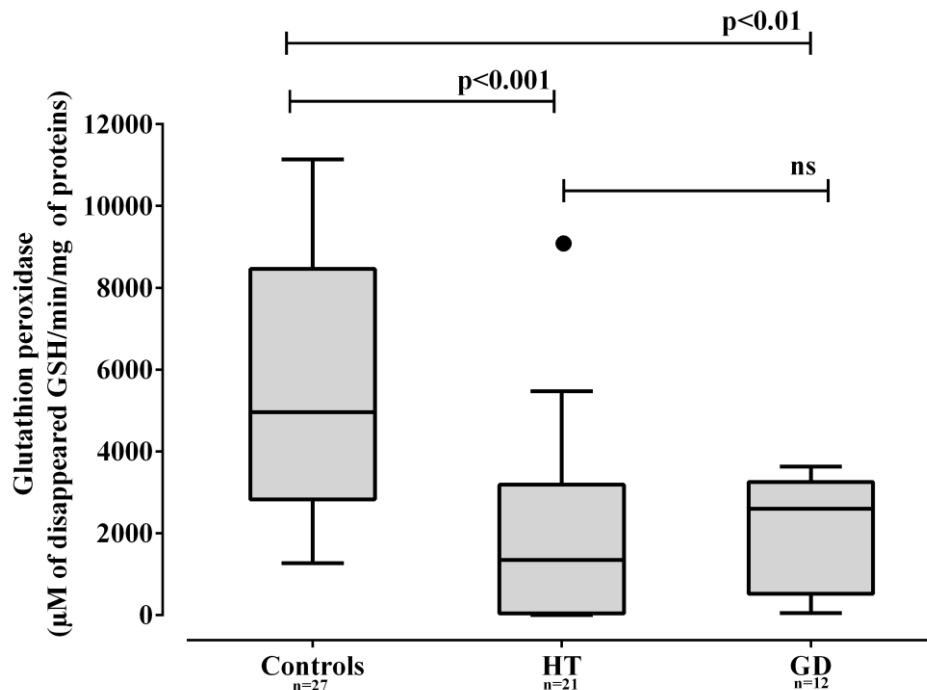


Fig 2: The selenium levels in the plasma of GD, HT patients and controls. The results are expressed as mean±S.E.M. Statistical analyses were performed using Tukey multi-comparison test, significant differences were found for p<0.05

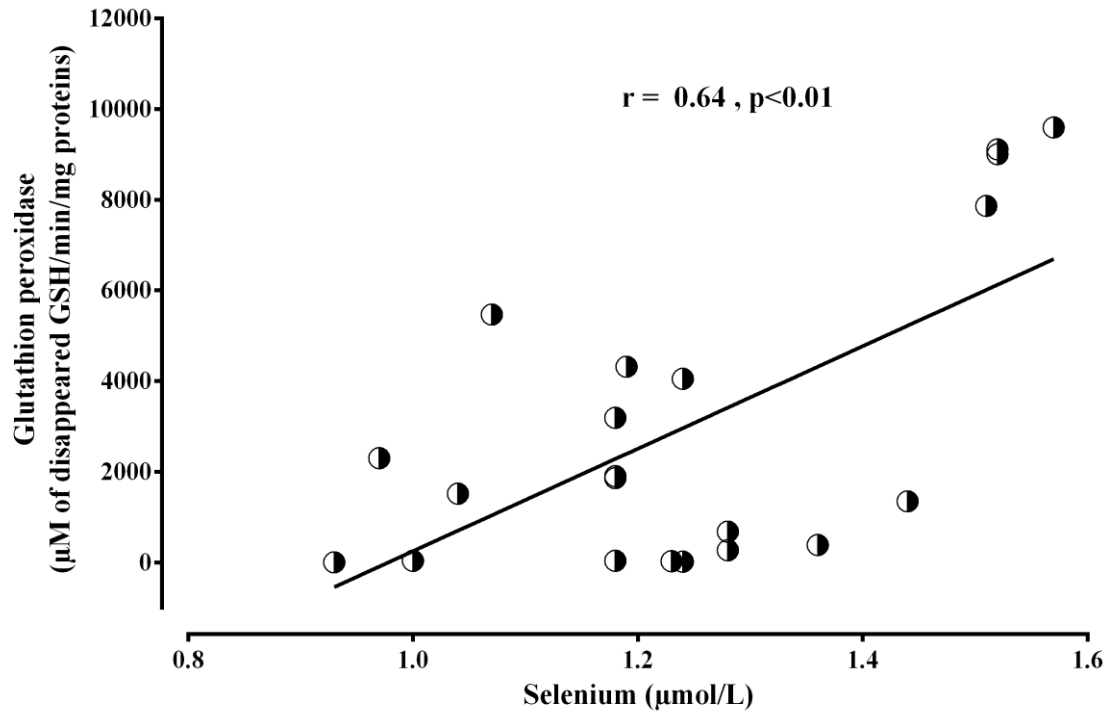


Fig 3: Correlation study between the selenium and the glutathione peroxidase levels in the plasma of HT patients. Statistical analyses were performed using the Pearson correlation test, significant differences were found for  $p < 0.05$ .

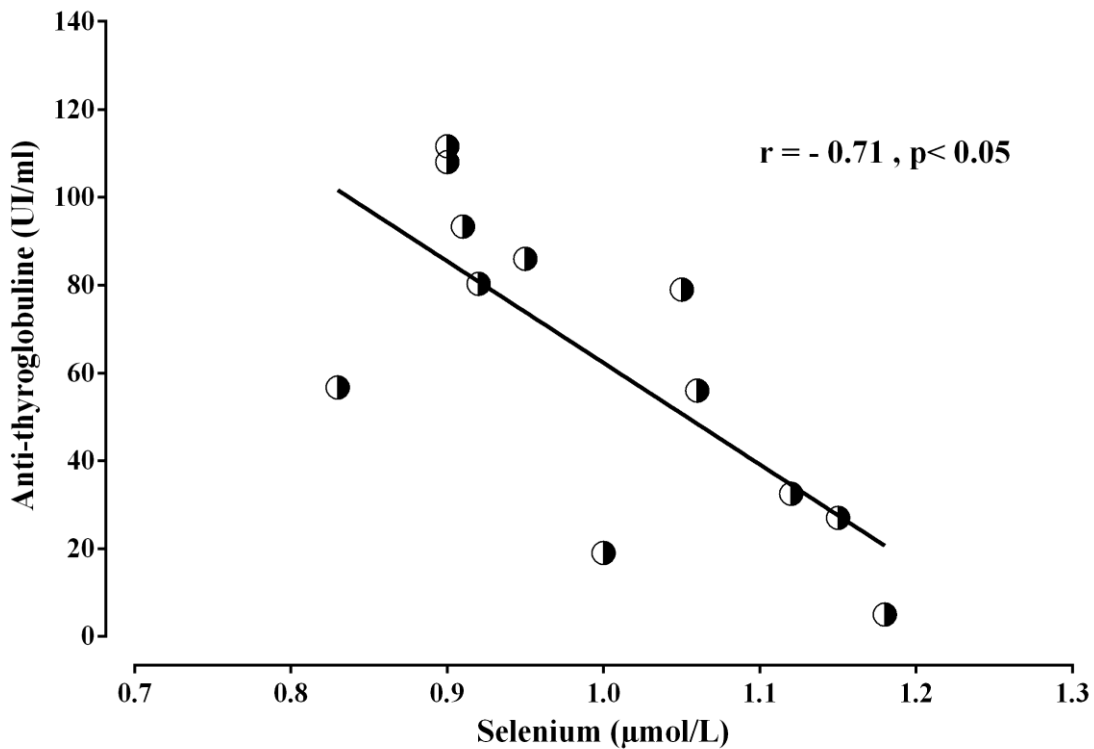
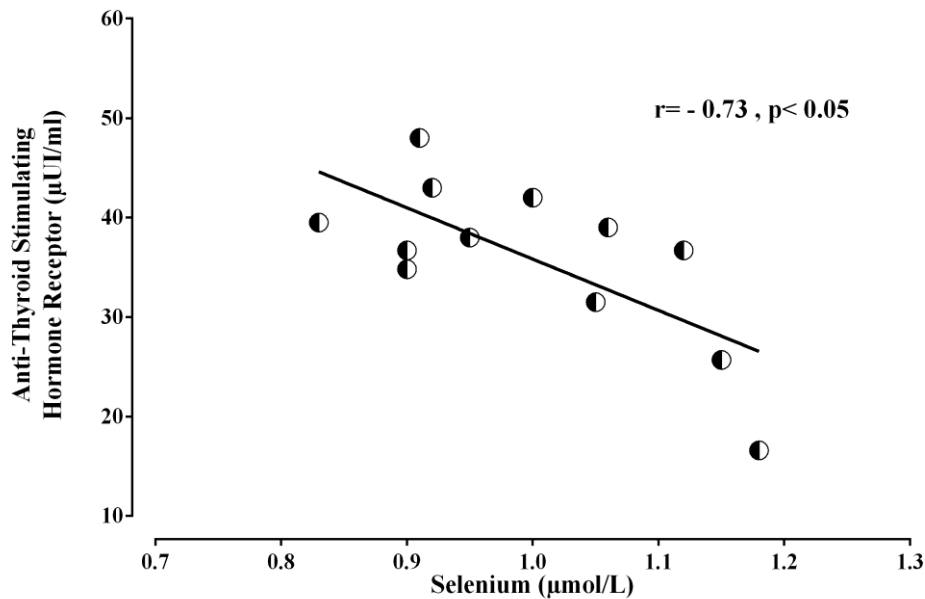


Fig 4: Correlation study between the selenium and the anti-Thyroglobuline antibody levels in the plasma of GD patients. Statistical analyses were performed using the Pearson correlation test, significant differences were found for  $p < 0.05$ .



**Fig 5: Correlation study between the selenium and the anti-Thyroid Stimulating Hormone Receptor antibody levels in the plasma of GD patients. Statistical analyses were performed using the Pearson correlation test, significant differences were found for  $p < 0.05$ .**

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