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Selenium and its relationship with selenoprotein P and glutathione peroxidase in children and adolescents with Hashimoto's thyroiditis and hypothyroidism

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ABSTRACT

The essential trace element selenium (Se) is required for thyroid hormone synthesis and metabolism. Selenoproteins contain selenocysteine and are responsible for biological functions of selenium. Glutathione peroxidase (GPx) is one of the major selenoproteins which protects the thyroid cells from oxidative damage. Selenoprotein P (SePP) is considered as the plasma selenium transporter to tissues. The aim of this study was to evaluate serum Se and SePP levels, and GPx activity in erythrocytes of children and adolescents with treated Hashimoto's thyroiditis, hypothyroidism, and normal subjects.

Blood samples were collected from 32 patients with Hashimoto's thyroiditis, 20 with hypothyroidism, and 25 matched normal subjects. All the patients were under treatment with levothyroxine and at the time of analysis all of the thyroid function tests were normal. GPx enzyme activity was measured by spectrophotometry at 340 nm. Serum selenium levels were measured by high-resolution continuum source graphite furnace atomic absorption. SePP, TPOAb (anti-thyroid peroxidase antibody), and TgAb (anti-thyroglobulin antibody) were determined by ELISA kits. T_4, T_3, T_3 uptake and TSH were also measured.

Neither GPx activity nor SePP levels were significantly different in patients with Hashimoto's thyroiditis or hypothyroidism compared to normal subjects. Although GPx and SePP were both lower in patients with hypothyroidism compared to those with Hashimoto's thyroiditis and normal subjects but the difference was not significant. Serum Se levels also did not differ significantly in patients and normal subjects. We did not find any correlation between GPx or SePP with TPOAb or TgAb but SePP was significantly correlated with Se.

Results show that in patients with Hashimoto's thyroiditis or hypothyroidism who have been under treatment with levothyroxine and have normal thyroid function tests, the GPx, SePP and Se levels are not significantly different.

1. Introduction

Thyroid hormones are key regulators of development, growth, differentiation, and many other physiological processes [1]. Among all human tissues, the thyroid gland contains the largest amounts of selenium (Se), and Se concentrations at this site are relatively stable irrespective of dietary intake and availability in the organism [2]. Se can exist in many different chemical forms in biological materials either as organic Se compounds, such as selenomethionine and dimethylselenide, and inorganic selenites and selenates [3]. Selenoproteins are unique as they contain selenium in their active

Abbreviations: Se, selenium; GPx, glutathione peroxidase; SePP, selenoprotein P; TPOAb, anti-thyroid peroxidase antibody; TgAb, anti-thyroglobulin antibody; T_3, triiodothyronine; T_4, tetraiodothyronine; TSH, thyroid-stimulating hormone.

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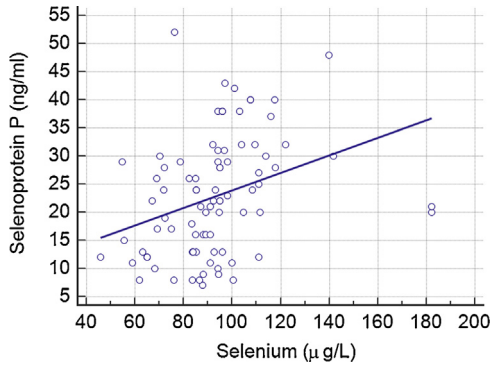

Fig. 1. Correlation between selenoprotein P levels and selenium levels ($r = 0.336$, $P = 0.02$).

site in the form of the 21st amino acid selenocysteine [4]. Selenium can also occur as selenomethionine, which is the main form of Se found in food. Animals cannot synthesize Se-meth or distinguish it from methionine and as a result it is nonspecifically incorporated into proteins as selenomethionine in the place of methionine and is not known to have a physiological function separate from that of methionine [5–8].

The thyroid contains several selenoproteins including iodothyronine 5′-deiodinase, glutathione peroxidase (GPx) which is part of the antioxidant defense mechanism against oxidative stress, thioredoxin reductase type 1, and selenoprotein P (SePP) [9]. Iodothyronine deiodinases are selenoproteins that catalyze the stereospecific and sequential removal of iodine atoms from the pro-hormone T_4, generating active and inactive isomers of both triiodothyronine (T_3) and diiodothyronine (T_2). This biotransformation of thyroid hormones occurs in practically every tissue [10].

The three steps of thyroid hormone biosynthesis are all catalyzed by a single enzyme, thyroid peroxidase (TPO). TPO uses H_2O_2 which is produced in high amounts by thyroid dual oxidases 1 and 2. Thus, in the thyroid, reactive oxygen species (ROS) and free radicals are constantly formed and participate in physiological and pathological processes in the gland, as a consequence of its normal physiological activity [11]. Cells have developed a comprehensive set of antioxidant defense mechanisms to limit the action of ROS. GPx acts as an efficient antioxidant enzyme by eliminating H_2O_2 and thus protects the thyroid cells from oxidative damage [12].

Selenoprotein P (SePP) represents the major selenoprotein in plasma [13]. SePP is an established marker for the nutritional selenium status [14]. Selenium deficiency reduces the plasma concentrations of SePP and selenium [15].

Se is crucial for thyroid gland functioning and thyroid hormone biosynthesis and metabolism [16]. On the other hand, thyroid hormone affects Se metabolism directly or indirectly and affects the serum selenium status and regulates the expression of several selenoproteins [17].

Furthermore, Se has been shown to be important in the regulation of immune function [18], and low selenium levels have been associated with poor immune function [19]. In some conditions, and especially in inflammatory diseases, selenium concentrations decline and selenoprotein biosynthesis is impaired [20]. Se treatment results in reduced inflammatory activity [18]. Severe and persistent selenium deficiency impairs thyroid hormone biosynthesis and also exaggerates destruction of follicular structures and their replacement by fibrotic tissue [18].

The human thyroid gland is the organ most affected by tissue-specific autoimmune diseases [18]. Autoimmune thyroid disease manifests itself in various clinical forms such as classical Hashimoto's thyroiditis. Hashimoto's thyroiditis is the most common form of thyroiditis in childhood and the most frequent cause of pediatric thyroid disease in iodine-replete areas of the world [21,22]. It has been hypothesized that nutritional selenium deficiency may promote the initiation or progression of thyroid autoimmunity [19]. Thus Se levels have been investigated in relation with thyroid disease and some of them have found lower Se levels in patients with autoimmune thyroid disorders. Selenium supplementation have also been examined in various clinical trials but the results have been equivocal [2,9,23–28]. These conflicting findings collectively indicate that the role of selenium and the benefit of its supplementation in the management of thyroid disorders are still unresolved [29].

Most of the studies have focused on the relationship between Se and autoimmune thyroid disorders and Se importance in non-autoimmune hypothyroidism has not been previously investigated. On the other hand, selenium status has not been studied in patients after long-term treatment of hypothyroidism and normalization of thyroid function. Thus, the present study was aimed at exploring the levels of Se and two important selenoproteins GPx and SePP in treated patients with Hashimoto's thyroiditis and non-autoimmune hypothyroidism and their comparison with normal subjects.

2. Subjects and methods

2.1. Subjects

Thirty five patients with Hashimoto's thyroiditis, 22 patients with hypothyroidism, and 30 normal subjects were randomly selected for the study. We tested the hypothesis that selenium is significantly different in patients with Hashimoto's thyroiditis or hypothyroidism compared with normal subjects at the 5% significance level. To detect such a difference, the sample size was calculated on the basis of the results of a previous study of selenium levels in patients with Hashimoto's thyroiditis [30] with 80% power and a confidence interval of 95% using PASS software (version 11). Therefore each study group was calculated to comprise 12 subjects per group. We enhanced the sample size in order to ensure that the required sample size would be achieved in case of missing data and to improve the power of the study.

The presence of Hashimoto's thyroiditis was documented by the increased levels of anti-thyroid peroxidase autoantibodies (TPOAb) and/or anti-thyroglobulin autoantibodies (TgAb). Autoantibodies are found in almost all of the patients with Hashimoto's thyroiditis, however TPOAb are more prevalent than TgAb [31]. Values in excess of 40 IU/mL for TPOAb and 125 IU/mL for TgAb were considered positive for the presence of these autoantibodies. Patients with hypothyroidism had been diagnosed based on elevated thyroid-stimulating hormone (TSH) and low T_4 levels, but normal TPOAb, and TgAb. Both groups of patients were under treatment with levothyroxine and at the time of sample collection they had normal TSH levels. The subjects who were taking any other medications especially those which could affect thyroid function or autoimmunity or Se levels (anti-depressive drugs, anti-psychotic drugs, corticosteroids, immunosuppressants, amiodarone, and preparations containing vitamins or trace elements) were excluded from the study. Case and control subjects were matches for age and body mass index (BMI).

The study was approved by the Ethics Committee of the Tehran University of Medical Sciences and all of the participants provided written informed consent.

2.2. Sample collection

Blood samples were collected after an overnight fast of 12 h. Blood samples for clinical chemistry analysis were clotted and

the serum was separated by centrifugation at 1200 × *g* for 15 min. Aliquots of serum were used for the measurement of hormones and the analyses of Se and SePP. An aliquot of blood specimen was drawn in heparin-containing tubes to separate erythrocytes. After centrifugation, separated erythrocytes were washed with saline and were kept frozen at −70 °C for later analysis of GPx.

2.3. Biochemical measurements

Serum total T_4 and T_3 levels were measured by RIA (Immunotech, Prague, Czech Republic). The concentrations of T_3 uptake, TSH, TPOAb and TgAb were measured by enzyme-linked immunosorbent assay (ELISA) (Monobind, CA, USA).

Glutathione peroxidase (GPx) activity was determined using a commercially available kit (Randox Laboratories Ltd., Crumlin, UK), based on the oxidation of glutathione by cumene hydroperoxide and utilization of NADPH by glutathione reductase. Hemolysate was prepared from erythrocytes. One milliliter of double-strength Drabkin's solution was added prior to enzyme assay, and measurements were performed within 20 min, at 340 nm. GPx activity was calculated according to the decrease in absorbance and expressed as U/g Hb. Intra- and inter-assay coefficients of variation (CV) were calculated and were 5.9 and 6.4%, respectively.

Serum SePP levels were measured using a human ELISA kit (USCN Life Science, Wuhan, China), according to the manufacturer's instructions. Intra- and inter-assay CV were calculated and were 6.3% and 7.6%, respectively.

2.4. Selenium measurement

All glassware and tubes used for serum sample preparation and analysis were acid-washed with nitric acid (10%) and rinsed thoroughly with deionized water. Serum selenium levels were measured by high-resolution continuum source graphite furnace atomic absorption using atomic absorption spectrometer ContrAA 700, (Analytik Jena AG, Jena, Germany). Serum samples were decomposed by treating them with nitric acid (65%) and hydrogen peroxide (30%) and heating at 130 °C for 2.5 h followed by mixing the solution with HCl (36%) and heating in 90 °C for 45 min. Serial dilutions of a 1000 ppm standard solution of selenium were also assayed along with samples. Seronorm trace element serum, level 2 L-2 (Lot No. 903107, SERO AS, Billingstad, Norway) was used for quality control in the beginning and during selenium measurement and the result of its selenium levels was 153.0 ± 9.0 (acceptable range 143–171 μg/L).

2.5. Statistical analysis

Normal distribution of data was analyzed by Shapiro–Wilk Test. Data are expressed as mean ± standard deviation (SD) for parametric variables and median (interquartile range) for non-parametric ones. Differences between groups were tested using analysis of variance (ANOVA) and Student *t*-test was used to compare the variables between cases and controls. Pearson correlation analysis was used to investigate correlation between parameters. Data were analyzed using SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). A *P*-value of less than 0.05 indicated statistical significance.

3. Results

Clinical and biochemical characteristics of studied subjects are summarized in Table 1. All the patients were under treatment with levothyroxine for a mean duration of 2.07 ± 0.45 years and at the time of sample collection, thyroid function tests were normal in all subjects. Only one of the subjects with Hashimoto's thyroiditis and one in hypothyroidism group had Se levels below the optimal level (54.4 μg/L and 55.3 μg/L, respectively) and Se levels in all the other subjects were within normal range according to previous studies (85.0 ± 10.8 μg/L for females and 83.7 ± 11.1 for males; reference range of 63–106 μg/L for the age of 1–16 years old) [32].

Se levels did not differ significantly between studied groups (Table 1). Neither GPx activity nor SePP levels were significantly different in various groups of patients compared to normal subjects (Table 1). Although there was a trend toward lower GPx and SePP levels in patients with hypothyroidism compared to those with Hashimoto's thyroiditis and normal subjects, but the difference was not significant (Table 1).

All the assayed parameters were compared between male and female subjects and there were no gender-related differences in Se, GPx and SePP. Correlations of Se, GPx, and SePP with T_3, T_4, T_3–T_4 ratio, TSH, TgAb and TPOAb were analyzed but the results were not significant. The assayed parameters were not significantly correlated with age. Se levels did not show any significant correlation with GPx but it had a significant correlation with SePP ($r = 0.336$, $P = 0.02$) (Fig. 1).

Table 1
Clinical and biochemical characteristics of studied subjects.

		Control($n = 30$)	Patients ($n = 52$)	
			Hashimoto's thyroiditis($n = 35$)	Hypothyroidism($n = 22$)
Gender	Male	8	4	6
	Female	24	31	14
Age (year)		12.4 ± 3.0	13.0 ± 4.2	11.4 ± 3.3
Height (m)		1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.1
Weight (kg)		43.8 ± 9.1	39.9 ± 13.6	39.6 ± 12.6
BMI		19.7 ± 2.8	19.7 ± 4.5	18.6 ± 4.2
T_4 (μg/dl)		9.3 ± 1.3	6.7 ± 2.7	10.0 ± 1.9
T_3 (ng/mL)		1.8 ± 0.4	1.7 ± 1.2	1.8 ± 0.5
T_3 uptake (%)		30.6 ± 9.3	28.4 ± 9.6	27.4 ± 11.5
TSH (μIU/mL)		2.3 ± 1.9	2.9 ± 2.8	6.3 ± 3.1
TgAb (IU/mL)		9.7 (1.3–16.2)	650.0 (136.0–2925.0)*	16.0 (0.5–35.6)
TPOAb (IU/mL)		2.8 (1.5–9.4)	557.0 (117.5–764.2)*	5.6 (4.2–24.8)
Se (μg/L)		97.2 ± 29.4	91.6 ± 17.7	85.9 ± 14.8
GPx (U/g Hb)		55.8 ± 12.9	54.7 ± 13.5	52.0 ± 12.6
SePP (ng/mL)		22.6 ± 12.0	24.2 ± 10.4	21.8 ± 10.0

Data are expressed as mean ± SD or median (interquartile range); BMI: body mass index, T_4: tetraiodothyronine, T_3: triiodothyronine, TSH: thyroid-stimulating hormone, TPOAb: anti-thyroid peroxidase anibody, TgAb: anti-thyroglobulin antibody, Se: selenium; GPx: glutathione peroxidase; SePP: selenoprotein P.

4. Discussion and conclusion

An individual's selenium status is an important parameter for general health and affects their risk of developing almost all major human diseases. Enzymes involved in thyroid hormone metabolism and regulation of redox state are selenoproteins and therefore selenium is important in thyroid gland function [20]. As a result, investigation of selenium status in thyroid disorders have been conducted in different studies and some of the results have indicated lower selenium levels in patients with Hashimoto's thyroiditis compared to healthy controls [30,33–34]. However, most of these studies have been carried out on patients with newly diagnosed thyroid disorders and with below normal Se levels.

In this study we investigated the selenium levels in serum of patients with Hashimoto's thyroiditis whose disease had been diagnosed and were under treatment with levothyroxine for mean duration of approximately 2 years and found no abnormality in selenium status compared to healthy controls and reference range for selenium.

Autoimmune thyroid diseases are generally characterized by an activated immune system and inflammation. Plasma concentrations of some micronutrients may be affected by systemic inflammation, which may confound the low plasma concentrations, indicating deficiency. Nevertheless, the main factor responsible for reduced plasma concentrations during the systemic inflammatory response seems to be selenium redistribution from the circulation to tissues that are involved in immune function [35]. Thus low levels of Se at the beginning of the thyroid disease may be the consequence of inflammation and not the cause of thyroid disease. It may also be possible that selenium role in thyroid disorders is more prominent in selenium deficient areas, and in selenium sufficient subjects, as in the current study, it may not be a cause of thyroid dysfunction.

In this study, Se levels were also investigated in patients with hypothyroidism and the results showed that selenium levels were normal in these patients. In accordance with these findings, the results of a study in US population showed that serum Se did not significantly affect the level of any thyroid variable [36].

Based on the importance of Se in thyroid function and some studies reporting low levels of Se in patients with thyroid disorders, Se supplementation has been considered in autoimmune thyroid disorders and several clinical trials have been performed. Nonetheless, the results are equivocal, with promising results in some studies and null effects in others [2,23–26,29]. Only one clinical trial has been conducted in children and found no beneficial effect from selenium supplementation in these subjects [37]. The discrepant results could not be explained neither from different settings of these experiments nor from the concomitant use of levothyroxine [19]. These clinical trials have been performed in conditions with marginally low selenium levels and it is not clear whether Se supplementation in Se-sufficient subjects is equally favorable [19]. Apparently addition of Se may be useful in patients with low Se intake as well as in those at the beginning of Hashimoto's thyroiditis. Conversely, Se supplementation is likely not to be beneficial in people with high serum Se concentrations [16]. The results of the present study emphasize this hypothesis.

GPx activity was also investigated in this study and was shown to be similar in patients and normal subjects. This may be the result of normal selenium status because glutathione peroxidase expression and activity is regulated by selenium and is affected by selenium deficiency [38].

Selenoprotein P is an important protein in selenium homeostasis and since its concentration falls in selenium deficiency, it can be used as an index of selenium nutritional status. However its levels and its relationship with Se status have not been previously investigated in thyroid disorders. Thus SePP levels was assessed in this study and no difference was observed between patients and normal subjects, however SePP showed a significant correlation with Se which shows that SePP can be used as a surrogate marker of selenium status. Since evaluation of Se levels is important before considering any Se supplementation and Se measurement is a relatively difficult and time consuming procedure, SePP assay provides a suitable alternative to Se assessment.

In conclusion, no significant difference in serum selenium was observed between children and adolescents with Hashimoto's thyroiditis or hypothyroidism whose thyroid function tests have been normalized by levothyroxine treatment. SePP levels and GPx activity which follow Se status were also similar in all the studied subjects.

Conflict of interest

The authors declare that there is no conflict of interest.

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