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ORIGINAL PAPER

THE ROLE OF SELENIUM, SELENOPROTEINS AND OXIDATIVE DNA DAMAGE IN *ETIOPATHOGENESIS* OF HASHIMOTO THYROIDITIS*

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Abstract

Selenoproteins and selenium (Se) are essential for thyroid hormone synthesis, metabolism and thyroid gland functions. The human thyroid gland is one of the organs vulnerable to tissue--specific autoimmune diseases. The aim of this study was to investigate roles of Se and several selenoproteins, including selenoprotein P (SePP), glutathione peroxidase-3 (GPx3), thioredoxin reductase (TrxR), type 1 iodothyronine deiodinase (DI1), selenoprotein W (SelW), selenoprotein H (SelH), and oxidative stress in etiopathogenesis of Hashimoto thyroiditis. A total of 40 patients with Hashimoto thyroiditis and 42 healthy controls were included in the study. Serum Se levels were measured by inductively coupled plasma optical emission spectrometry (ICP-OES). 8-hydroxydeoxyguanosine (8-OHdG), SePP, SelW, SelH, GPx-3, TrxR, and DI1 levels were determined by enzyme-linked immunosorbent assay (ELISA) kits. Se levels were significantly decreased, but plasma SelH, 8-OHdG levels, and TrxR activities were significantly increased in the Hashimoto thyroiditis group. Plasma SePP levels, GPx3and DI1 activities did not signifi-

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cantly changed in Hashimoto thyroiditis patients. Changes in circulating Se and selenoprotein levels/activities, together with increased oxidative stress, might have important impact on the etiopathogenesis of Hashimoto thyroiditis.

Keywords: Hashimoto thyroiditis, selenium, selenoprotein P, selenoprotein W, selenoprotein H, glutathione peroxidase-3, thioredoxin reductase, type 1 iodothyronine deiodinase, 8-hydroxydeoxyguanosine.

INTRODUCTION

Thyroid glands synthesize thyroid hormones (TH), which are important regulators of physiological processes, such as body temperature, heart activity and energy metabolism (Mullur et al. 2014). The thyroid gland is also one of the tissues with the highest selenium (Se) content in the human body (Schmutzler et al. 2007). Se and some selenoproteins are involved in the thyroid hormone biosynthesis and metabolism (Brown, Arthur 2001). A group of selenoproteins function in the thyroid gland, such as deiodinases (DIs), glutathione peroxidases (GPx), thioredoxin reductase (TrxR), and selenoprotein P (SePP) – (Behne et al. 1988). Type 1 and 2 DIs are expressed in thyroid tissue, and are responsible for the deiodination process of thyroxine, the major thyroid hormone secreted by the thyroid gland. GPxs and TrxR protect the thyroid gland against oxidative stress by eliminating H_2O_2, which is produced in large quantities during thyroid hormone biosynthesis. SePP is the main selenoprotein responsible for the transport of Se in the circulation. Due to this function, it is considered to be the best nutritional biomarker for Se (Xia et al. 2010). It also has antioxidant activity by reducing hydroperoxides, and some regulatory functions towards metabolism (Misu et al. 2010, Kurokawa et al. 2014). Thyroid peroxidase (TPO) catalyzes all three steps of thyroid hormone biosynthesis, in which high amounts of H_2O_2 are produced and used (Schmutzler et al. 2007). Thyroid tissue should have powerful antioxidant defense mechanisms to limit the action of H_2O_2 and other radical species produced as a consequence of its physiological activity. Selenoproteins play major roles in redox-regulatory processes by their oxidoreductase activities, although some selenoproteins exert their own unique functions, such as Se transport, inflammatory, and immune responses, cell cycle regulation and apoptosis, and synthesis of thyroid hormones (Powis et al. 1997, Hawkes, Alkan 2010, Huang et al. 2012, Schomburg 2012, Hariharan, Dharmaraj 2020).

There are two main groups of mammalian selenoproteins according to the location of selenocysteine (Sec). In one group of selenoproteins, Sec localizes in a site very close to the C terminus of protein; TrxRs and selenoprotein K (SelK) are included in this group. In the other group, Sec localizes at the N terminal of selenoprotein; examples include GPxs, DIOs, selenopro-

tein H (SelH) and selenoproteinW (SelW) (Lu and Holmgren 2009). SelH has thioredoxin-like redox fold suggesting that it functions in the cellular redox homeostasis (Dikiy et al. 2007). By its peroxidase activity and DNA binding function, SelH acts against oxidative stress in the nucleus (Zhang et al. 2019).

Among the mammals, highly conserved SelW has an important role in the health of skeletal and cardiac muscle in humans. According to the literature, SelW is an S-glutathionylated protein, suggesting that it has a redox function (Whanger 2009). Both SelH and SelW are the Se-sensitive selenoproteins. Their expression is impaired in states of Se deficiency (Zhang et al. 2019).

The autoimmune diseases of the human thyroid gland, such as Hashimoto's thyroiditis (HT), are among the most common autoimmune conditions in humans (Vanderpump et al. 1995). Se deficiency is postulated as a triggering and promoter factor for thyroid autoimmunity (Effraimidis, Wiersinga 2014). Several studies reported that Se deficiency impairs GPx activity and induces apoptotic cell death by increasing H_2O_2 (Žarković 2012).

Se is essential for physiology of the thyroid while the thyroid gland itself seems to regulate metabolism of Se and selenoproteins. Exact etiology of the immune response to thyroid tissue remains unknown in HT. The aim of this study was to investigate the role of the serum Se, some selenoproteins like GPx-3, TrxR and DI1, SePP, SelH, SelW, and oxidative stress in etiopathogenesis of HT.

MATERIAL AND METHODS

Study groups

Forty patients with HT (26 females/14 males, age 21-56 median 38) and 42 healthy controls (27 females/15 males, age 24-60, median 39) were included. A protocol of screening and diagnosing HT was developed from the guidelines and protocols of the Department of Internal Medicine, Sakarya University Medical Faculty. The patients were selected based on an enlarged thyroid, characteristic ultrasound signs (hypoechogenicity and non-homogeneous texture) and a high level of either anti-thyroid peroxidase (normal range in the method used: 0-34 IU ml^{-1}) or anti-thyroglobulin (normal range in the method used: 0-115 $IUml^{-1}$), with or without clinical and biochemical hypothyroidism. An Abbott I2 000 SR immunology assay analyzer (Abbott Laboratories, Abbott Park, IL, USA) was used for thyroid function tests. Diagnoses were confirmed by fine needle aspiration cytology in controversial cases. To control potentially confounding factors, participants with a reported history of thyroid cancer and/or previous thyroid surgery were excluded. Also, exclusion criteria were the presence of any comorbid cardiac, autoim-

mune, infectious, musculoskeletal or malignant disease, or a recent history of operation or trauma, the use of antioxidant and minerals supplements. The healthy controls had normal thyroid functions, normal thyroid ultrasound as well as negative thyroid autoantibodies. All patients were treated with thyroid hormone replacement therapy after blood sampling. Levo--Thyroxine 4 (L-T4) (1.6-1.8 μg kg × g) was administered orally for normal values of circulating thyrotropin (TSH) levels to the HT patients. All participants were informed about the survey and freely signed and dated the consent form. The protocol was approved by the Ethics Committee of Sakarya University Medical Faculty, and was implemented in accordance with the Declaration of Helsinki (71522473/050.01.04/50).

Peripheral blood samples were collected in tubes with or without ethylenediaminetetraacetic acid (EDTA) after overnight fasting. The blood samples were centrifuged at 1500 × g for 20 min at room temperature. Blood serum and plasma were separated and stored at −80°C until further assays for Se, SePP, SelW, SelH, 8-OHdG levels and GPx-3, TrxR and DI1 activities.

Measurement of selenium levels

Serum Se levels were measured by inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo iCAP 6000, *Thermo Fisher Scientific, USA).* All reagents were of analytical reagent grade. High purity deionized water obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used throughout. All glassware was cleaned by immersion in 20% (v/v) HNO_3 for 48 h, washed three times with ultrapure water, and then dried before use. Distilled water containing 0.3% HNO_3 in Se standard solution at concentrations of 5, 10, 50, 250, and 500 μg L^{-1} using a stock solution of 1000 mg L^{-1} (Millipore, Bedford, MA, USA). The blood serum Se levels were determined by ICP-OES using a spectrometer after diluting each sample with 3 mL of 1% HNO_3 (65%) and 1 mL of 1.5% $HClO_4$ (70%) and obtaining the total volume of 25 mL with ultrapure water. Se analytical parameters for ICP-OES, which are frequency, power, coolant, auxiliary, nebulized argon gases and nebulizer pressure, sample flow rate, integration time and plasma position, were optimized. The analytical lines of ICP-OES determinants in Se analyses were 196.026 nm. Results were calculated as μg L^{-1} in serum samples.

Measurement of selenoprotein P, selenoprotein W, selenoprotein H, 8-hydroxydeoxyguanosine levels and glutathione peroxidase-3, thioredoxin reductase and type 1 iodothyronine deiodinase 1activities

Plasma SePP, SelW, SelH**,** 8-OHdG levels and GPx-3, TrxR, and DI1 activities were measured by the enzyme-linked immunosorbent assay (ELISA) using commercially available kits (YeHua Biological Technology Co., Ltd. Gical Technology Co., Ltd, Shanghai, China), in accordance with the manu-

facturers' instructions. The intra-assay and inter-assay variabilities of the ELISA kit for SePP, SelW, 8-OHdG levels and GPx-3, TrxR, and DI1 activities were 5.1 and 5.9%; 5.5 and 6.1%; 4.7 and 5.3%; 7.3 and 8.4%; 8.1 and 8.8%; 5.7 and 7.5%, respectively.

Statistical analysis

Statistical analysis was performed using SPSS 17.0 statistical software for Windows (SPSS, Chicago, IL, USA). Results were reported as mean±SD and median (min-max values). The means for normally distributed continuous variables were compared by the Independent Samples *T*-test. Skew--distributed continuous variables were compared using the Mann-Whitney *U*-test. The correlations between Se, SePP, SelH, SelW, 8-OHdG levels and GPx-3, TrxR, and DI1 activities were assessed by using the Pearson's correlation coefficient. The significance level of $P<0.05$ was accepted.

RESULTS

Data from a total of 82 subjects, 40 with HT patients and 42 healthy subjects, were included in this study. The median age of the patient and control groups did not differ significantly between patients and controls ($P>0.05$). Table 1 presents the characteristics of variables studied, including the levels of Se, SePP, SelH, SelW, 8-OHdG, the activities of GPx-3, TrxR, and DI1. There were no significant differences between HT patients and healthy subjects regarding SePP ($P=0.072$), SelW ($P=0.123$), DI1 ($P=0.173$),

Table 1

Comparison of Se, SelH, SePP, SelW, 8-OHdG levels and DI1, TrxR and GPx-3 activities between patients with Hashimoto thyroiditis and control groups

Specification	Control group n:42	Hashimoto thyroiditis group n:40	*P* value
Se (μg L^{-1})	169.40±21.80	148.90±32.30	**0.002**
SelH (μg L^{-1})	39.29±11.36	47.02±12.61	**0.007**
SePP (μg L^{-1})	30.18±5.53	32.06±5.34	0.072
TrxR (μg L^{-1})	10.41±2.87	12.87±3.77	**0.002**
SelW (μg L^{-1})	99.02 (8.67-122.81)	112.64 (57.29-144.02)	0.123
8-OHdG (μg L^{-1})	25.77 (1.31-39.97)	28.13 (13.80-50.68)	**0.004**
DI1 (μg L^{-1})	47.10 (8.31-132.50)	72.90 (36.13-129.92)	0.173
GPx-3 (μg L^{-1})	0.0307 (0.0116-0.1244)	0.0348 (0.0165-0.0533)	0.785

Data are presented as mean ± SD, median (min-max). Bold values indicate statistical significance. Se – selenium, SelH – selenoprotein H, SePP – selenoprotein P, TrxR – thioredoxin reductase, SelW – selenoprotein W, 8-OHdG – 8-hydroxydeoxyguanosine, GPx3– glutathione peroxidase-3, DI1 – type 1 iodothyronine deiodinase

and GPx-3 (P=0.785). Serum levels of Se were decreased in HT patients compared to the control group (P=0.002). The levels of SelH, 8-OHdG and activities of TrxR were found to be higher in HT patients compared to the control group (P=0.007, P=0.004 and P=0.002, respectively).

To further explore the relationships between Se, SelH, SePP, SelW, 8-OHdG, GPx-3, TrxR, and DI1, correlation analyses were performed (Table 2). Plasma SePP levels showed a positive correlation with SelH, SelW,

Table 2

Correlation of Se, SelH, SePP, SelW, 8-OHdG levels and DI1, TrxR and GPx-3 levels (μg L^{-1}) of patients with Hashimoto thyroiditis

Specification	Se	SelH	SePP	SelW	TrxR	8-OHdG	DI1
SelH	r=0.050 P=0.791						
SePP	r=-0.086 P=0.650	**r=0.761** **P=0.001**					
SelW	r=0.011 P=0.951	**r=0.593** **P=0.001**	**r=0.678** **P=0.001**				
TrxR	r=0.035 P=0.855	**r=0.517** **P=0.002**	**r=0.443** **P=0.011**	**r=0.401** **P=0.023**			
8-OHdG	r=-0.259 P=0.212	r=0.367 P=0.071	**r=0.478** **P=0.016**	**r=0.432** **P=0.024**	**r=0.451** **P=0.024**		
DI1	r=-0.114 P=0.536	**r=0.662** **P=0.001**	**r=0.612** **P=0.001**	**r=0.609** **P=0.001**	**r=0.509** **P=0.003**	**r=0.630** **P=0.001**	
GPx3	r=-0.271 P=0.133	**r=0.662** **P=0.001**	**r=0.750** **P=0.001**	r=0.306 P=0.078	**r=0.447** **P=0.010**	**r=0.443** **P=0.021**	**r=0.797** **P=0.001**

Values r – correlation coefficient is given, P-values (P<0.05) are accepted as significant (Pearson correlation)

8-OHdG levels, TrxR, DI1 and GPx-3 activities. Additionally, plasma SelH levels showed a positive correlation with SelW, TrxR, DI1, and GPx-3. Moreover, plasma SelW were found to positively correlate with 8-OHdG levels, TrxR and DI1 activities. Also, plasma TrxR activities were found to positively correlate with 8-OHdG levels as well as DI1 and GPx-3 activities. Plasma 8-OHdG levels showed a positive correlation with DI1 and GPx-3 activities. Furthermore, plasma DI1 activities were found to correlate with positively GPx-3 activities in patients with HT.

DISCUSSION

Thyroid tissue is one of the most abundant selenoprotein-containing tissues in the human body. Expectedly, essential trace element Se and some selenoproteins are important in thyroid function, and play a role in some of its physiological and pathological conditions. Autoimmune thyroid disease (AITD), such as HT, is relatively common form of thyroiditis (Vanderpump et al. 1995). In this study, levels of Se, some selenoproteins, and oxidative stress values were evaluated in the patients and healthy controls in order to understand their roles in the etiopathogenesis of HT.

In our study, we found that the serum Se levels were significantly lower in HT patients compared to control. Plasma SelH, 8-OHdG levels, and TrxR activities were increased, whereas plasma SePP levels as well as plasma GPx3 and DI1 activities were not significantly changed in HT patients.

Relationships of Se levels with thyroid pathologies have been investigated before, as Se is an essential trace element for thyroid functions, like the deiodination process of thyroxine and degradation of excessive generated H_2O_2. Low levels of Se have been reported in patients with autoimmune thyroid disorders (Rasic-Milutinovic et al. 2017, Mehl et al. 2020). Consistent with these studies, Se levels were significantly lower in HT patients compared to control group in our study. Contrary to our findings, several studies reported no change in Se levels in patients with autoimmune thyroid disorders (Nourbakhsh et al. 2016, Federige et al. 2017). This might be due to the characteristics of different research group, for example tests were carried on children and adolescents who were under treatment with levothyroxine and had normal thyroid function tests. Since the thyroid gland itself is also a regulating factor towards the metabolism of Se and selenoproteins, the above factors might have had an impact on the reported findings (Mittag et al. 2010). Although they found unchanged Se levels, SePP levels, which are a biological marker for Se levels in the body, were either lower or unchanged in their studies. In our study, plasma SePP levels as well as GPx3 and DI1 activities were not significantly changed but plasma TrxR activities were increased in HT patients. In the literature, there are conflicting results regarding alterations of selenoproteins related with thyroid functions, suggesting some influence of the conditions such as the status of thyroid hormones in patients or the actual course of treatment of the disease.

We found slightly higher Se levels in both patients and controls in our study compared to previous studies reporting plasma Se concentrations in the range of 60 and 120 μg L^{-1} to be normal (Drutel et al. 2013). We do not know the exact reason why Se in both HT and control groups were higher in our study, but it might be due to the Se content of soil and daily Se intake in our region (Karadas 2014). Although patient and control groups showed mildly higher levels of Se compared to those given in the literature, our study patients' Se levels were significantly lower than the controls, suggest-

ing that decreasing levels of Se resulted from the course of the disease, probably from the inflammatory process of AITD. Due to the inflammatory process, down-regulation of the hepatic biosynthesis may result in the negative acute phase regulation of serum Se levels (Schomburg 2012). This may also be an explanation for the unchanged SePP level and GPx and DI1 activities in the patient group. This amount of decrease in Se levels may not result in impaired synthesis of these selenoproteins. It is clear that the decrease in Se levels seem to be involved in the pathogenesis of HT.

We also investigated changes in other selenoproteins that are not directly related to thyroid function but sensitive to Se levels in the patients with HT. We selected SelH and SelW for this purpose as they are the most sensitive ones to a decrease in Se levels (Zhang et al. 2019). These selenoproteins are not among the ones expressed in the thyroid tissue. We found that SelH levels were significantly increased in HT patients, while SelW did not statistically differ between the HT and control groups. In fact, SelW displayed binomial distribution in both HT patients and controls. Eighty-five percent of the patients were at the high peak, while sixty-five percent of the controls were at the high peak (data were not presented), indicating a tendency of higher values in patients compared to controls. However, we may not have been able to detect the existing change in SelW due to its wide and binomial distribution. We do not know the mechanism and function of this marked increase of SelH in HT patients, but suggest that it may be related to its antioxidant properties. It might also be an antioxidant response to increased oxidative stress in HT. We also measured oxidative stress in our study by measuring 8-OHdG levels. In agreement with our findings, some studies previously concluded that over-expression of some selenoproteins under the increased oxidant stress conditions conferred resistance to oxidant stress in cells (Whanger 2009).

In conclusion, changes in circulating Se and selenoproteins levels/activities together with increased oxidative stress seem to have an important role in the pathogenesis of HT. It remains unclear whether decreased serum Se levels is a consequence of the disease state or the real reason of autoimmune/pro-inflammatory conditions. Also, we must take into consideration that plasma Se levels may not reflect thyroid Se levels. Further studies are required on the thyroid gland tissue and blood fraction for elucidating the exact roles of Se and selenoproteins in HT.

Compliance with ethical standards

Ethical approval

The study protocol was previously reviewed and approved by the Ethics Committee of the University of the Sakarya, Faculty of Medicine (71522473/050.01.04/50). All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Contribution

FBSC, DAC, GCC, and BA researched literature and conceived the study. FBSC, DAC, GCC, SD, NB and BA were involved in protocol development, gaining ethical approval, patient recruitment and data analysis. FBSC, DAC, GCC and BA wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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