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ORIGINAL ARTICLE

Evaluation of oxidative status in patients with hyperthyroidism

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Abstract Data on the antioxidant levels enzyme in patients with hyperthyroidism are limited and conflicting. Therefore, the objective of this study was to evaluate the oxidative status using an automated method in patients with hyperthyroidism. Thirty-six subjects with hyperthyroidism and 30 healthy controls were enrolled in this study. Serum oxidative status was determined via measurement of total antioxidant capacity (TAC) and total oxidant status (TOS) and calculation of oxidative stress index (OSI). Serum TAC levels were significantly lower in patients with hyperthyroidism than controls ($P = 0.002$), while serum TOS levels and OSI values were significantly higher ($P = 0.008$, 0.004; respectively). Serum TAC levels were correlated with TSH levels (rho = 0.223, $P = 0.032$), FT3 levels (rho = −0.434, $P = 0.002$) and FT4 levels (rho = −0.363, $P = 0.003$) in patients. Further, TOS levels and OSI values were correlated with TSH levels (rho = −0.245, $P = 0.037$; rho = −0.312, $P = 0.011$, respectively), FT3 levels (rho = 0.293, $P = 0.017$, rho = 0.505, $P = 0.002$, respectively), and FT4 levels (rho = 0.302, $P = 0.006$, rho = 0.321, $P = 0.008$, respectively) in patients. Duration of disease was significantly correlated with OSI values in patients (rho = 0.420, $P = 0.011$), while no correlation with serum TAC levels and TOS levels ($P > 0.05$). Oxidants are increased and antioxidants are decreased in patients with hyperthyroidism; as a result, the oxidative–antioxidative balance is shifted to the oxidative side. Increased oxidative stress may play a role in the pathogenesis of hyperthyroidism. It is believed that supplementation of antioxidant vitamins such as vitamins C and E may be helpful for these patients.

Keywords Hyperthyroidism · Total oxidant status · Total antioxidant capacity · Oxidative stress index

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Introduction

Thyroid hormones (thyroxine T4 and triiodothyronine T3) are involved in the regulation of numerous body functions including lipid and carbohydrate metabolism, oxygen consumption, and several physiological functions such as development, reproduction, and growth [1]. Also, thyroid hormones act on mitochondria by regulating the energy metabolism and mitochondrion which is a major source of free radicals in the cell [2]. The thyroid gland is the body's primary regulator of metabolism. Thyroid stimulating hormone (TSH) affect metabolism and may be affected by the thyroxine secretions. High concentrations of thyroid hormones affect oxygen metabolism and stimulate formation of free radicals in mitochondria [3]. In the course of hyperthyroidism, oxidative stress and the peroxidation of lipids can be generated [4].

Thyroid dysfunctions are associated with many pathological signs in the body. There are many reports that showed increased lipid peroxidation reactions and reactive oxygen species (ROS) in thyroid dysfunctions [5, 6]. On the other hand, hyperthyroidism is characterised by an

increasing cellular metabolic rate, and thus an increased amount of free radicals [7, 8]. Hyperthyroidism enhances ROS generation and produces changes in various tissue antioxidant systems, which participate in the development of hyperthyroidism-induced tissue damage [9, 10]. ROS play an important role in physiological mechanisms, but extremely reactive types also cause oxidative damage in molecules [11].

The presence of the following antioxidative enzymes in the thyroid gland has been documented: superoxide dismutase (SOD) [12, 13], catalase (CAT) [14], and glutathione peroxidase (GPX) [15]. However, clinical investigations of the disturbed antioxidative defense system in hyperthyroidism are scarce and conflicting [16–20].

The measurement of total antioxidant capacity (TAC) and total oxidant status (TOS) was useful tests for prediction of oxidative status [21]. To the best of our knowledge, the oxidative status in patients with hyperthyroidism have not been reported yet. Therefore, the objective of this study was to evaluate oxidative status via measurement of TAC and TOS and calculation of oxidative stress index (OSI) in patients with hyperthyroidism using an automated method.

Materials and methods

The study was performed on 36 patients and 30 healthy control subjects. Twenty-one patients were diagnosed as Basedow disease, 15 as toxic multinodular goiter. The patients were diagnosed according to FT3, FT4, and TSH values and thyroid Doppler ultrasonography and scintigraphy. The average duration of disease in patients with hyperthyroidism was 2.27 ± 1.46 months. All of the controls were euthyroid and did not have any significant medical condition.

The study protocol was carried out in accordance with the Helsinki Declaration as revised in 1989 and approved by the local research committee for ethics. All subjects were informed about the study protocol and written consents were obtained from all participants.

Exclusion criteria

History of alcohol abuse, smoking habit, intravenous drug abuse, pregnancy, antioxidant supplements (vitamin E, β-carotene, ascorbic acid, glutathione, and probucol), hypertension, diabetes mellitus, liver or pulmonary disease, rheumatoid arthritis, renal disease, and coronary heart disease were excluded from the study.

Blood sample collection

A blood sample was collected from each subject while fasting. Venous blood samples were collected into empty tubes a 10 ml and immediately stored on ice at 4°C. Following serum separation by centrifugation at 3000 rpm for 10 min, one aliquot was used freshly for thyroid function tests. Remaining serum portions for measurement of TOS and TAC levels were stored at −80°C until they were used.

Measurement of serum TAC

Serum TAC was determined using a novel automated measurement method, developed by Erel [22]. In this method, hydroxyl radical, which is the most potent biological radical, is produced. In the assay, ferrous ion solution, which is present in the Reagent 1 is mixed by hydrogen peroxide, which is present in the Reagent 2. The sequential produced radicals such as brown colored dianisidinyl radical cation, produced by the hydroxyl radical, are also potent radicals. Using this method, antioxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The assay has got excellent precision values lower than 3%. The results are expressed as mmol Trolox equiv/l.

Measurement of serum TOS

The TOS of serum was determined using a novel automated measurement method, developed by Erel [23]. Oxidants present in the sample oxidize the ferrous ion–*o*-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equiv/l).

OSI

The percent ratio of the TOS to the TAC gave the OSI, an indicator of the degree of oxidative stress [24]. OSI (arbitrary unit) = TOS (μmol H_2O_2 equiv/l)/TAC (mmol Trolox equiv/l).

Other parameters

Free T3 (FT3), free T4 (FT4), and TSH values were measured with chemiluminescence method (E-170 hormone auto-analyser Roch diagnostic system). The reference range was 1.82–4.62 pg/ml for FT3, 0.93–1.71 ng/dl for FT4, and 0.27–420 μIU/ml for TSH.

Statistical analysis

Data were presented as median and range. Continuous variables were compared using Student's *t* test. Nonparametric continuous variables were compared by the Kruskal–Wallis one-way analysis of variance with post hoc analysis using a Mann–Whitney *U* test. Fisher's exact test was used to test the sex differences between groups. Correlation analyses were performed using Spearman's correlation test. $P < 0.05$ was considered as statistical significance.

Results

Demographic and clinical data of the subjects are shown in Table 1. There were no significant differences between hyperthyroidism subjects and controls in respect to age, gender, and body mass index (BMI) (all $P > 0.05$). The average duration of disease in patients with hyperthyroidism was 2.27 ± 1.46 months.

Serum TSH levels were significantly lower in patients with hyperthyroidism than controls ($P = 0.010$), while serum FT3 levels and FT4 levels were significantly higher ($P = 0.012$, 0.008, respectively) (Table 1).

Table 1 Demographic and clinical parameters in patients with hyperthyroidism and healthy controls

Parameters	Hyperthyroidism ($n = 36$)	Control ($n = 30$)	*P*
Age (years)	22 (28–39)	35 (25–45)	0.085
Sex (female/male)	20/16	18/12	0.218
BMI (kg/m^2)	21.4 (17.1–24.5)	22.5 (20.5–27.2)	0.125
TSH (μIU/ml)	0.02 (0.0–0.13)	1.02 (0.25–2.65)	0.010
FT3 (pg/ml)	6.19 (4.58–7.8)	2.33 (1–3.87)	0.012
FT4 (ng/dl)	2.55 (1.85–4.37)	1.28 (0.37–2.66)	0.008

BMI body mass index

Data were presented as median and range

Serum TAC levels were significantly lower in patients with hyperthyroidism than controls ($P = 0.002$), while serum TOS levels and OSI values were significantly higher ($P = 0.008$, 0.004, respectively) (Table 2). However, there were no differences in TAC levels, TOS levels, and OSI values between basedow disease and toxic multinodular goiter groups ($P > 0.05$).

Serum TAC levels were correlated with TSH levels (rho = 0.223, $P = 0.032$), FT3 levels (rho = −0.434, $P = 0.002$), and FT4 levels (rho = −0.363, $P = 0.003$) in patients. Further, TOS levels and OSI values were correlated with TSH levels (rho = −0.245, $P = 0.037$; rho = −0.312, $P = 0.011$, respectively), FT3 levels (rho = 0.293, $P = 0.017$, rho = 0.505, $P = 0.002$, respectively), and FT4 levels (rho = 0.302, $P = 0.006$, rho = 0.321, $P = 0.008$, respectively) in patients.

Duration of disease was significantly correlated with OSI values in patients (rho = 0.420, $P = 0.011$), while no correlation with serum TAC levels and TOS levels ($P > 0.05$).

Discussion

In this study, we assayed oxidative status of the study population using TOS and TAC along with calculation of OSI, an indicator of oxidative stress, reflects the redox balance between oxidation and antioxidation. In this study, we observed that hyperthyroidism subjects had decreased TAC levels along with increased TOS levels and OSI values. However, there were no differences in TAC levels, TOS levels, and OSI values between basedow disease and toxic multinodular goiter groups ($P > 0.05$). Thus, the potency of the oxidative stress is significantly related to the severity of hyperthyroidism. The increase in oxidative stress that was observed in hyperthyroidism subjects of this study resulted from both increase in oxidants and decrease in antioxidant. To the best of our knowledge, this is the first study, oxidative status in hyperthyroidism subjects was determined using measurement of TAC along with measurement of TOS level and calculation of OSI.

In this study, we used an automated method, which has several major advantages in comparison with other currently available methods, to measure TAC in study population. The novel assay is a simple and cheap, and can easily be fully automated method. Further, the developed method has high linearity and the results are highly reproducible, and do not interact with commonly occurring serum components such as bilirubin, serum lipids, and anticoagulants. Accurate measurements of TAC can be obtained as little as 10 min, making this assay eminently suitable for the clinical biochemistry laboratory [25].

Table 2 Oxidative and antioxidative parameters in patients with hyperthyroidism and healthy controls

Parameters	Hyperthyroidism ($n = 36$)	Control ($n = 30$)	P
TAC (mmol Trolox Equiv/l)	1.72 (0.85–2.72)	2.12 (1.23–4.36)	0.002
TOS (μmol H_2O_2 Equiv/l)	4.94 (2.56–7.58)	4.12 (1.31–8.71)	0.008
OSI (arbitrary unit)	3.03 (1.11–5.89)	2.09 (0.57–4.63)	0.004

TAC total antioxidant capacity; *TOS* total oxidant status; *OSI* oxidative stress index

Data were presented as median and range

Thyroid hormones increase oxygen consumption via a thermogenetic effect. In hyperthyroidism caused by thyroxine or triiodothyronine administration, the increase in metabolic rate together with the increase in oxygen consumption enhances microsomal oxidative capacity and free radical formation. There are conflicting results about an increase or decrease in the activities of antioxidant enzymes in hyperthyroidism [16–20, 26–29]. In some studies, it has been reported that SOD activity was significantly increased [18, 26, 28]. On the contrary, several authors reported that SOD activity were reduced in patients with hyperthyroidism [27, 29].

Recently, increasing experimental and clinical studies have shown that free radicals play a key role in the etiology of many diseases. Thyroid hormones cause oxidative stress as they increase ROS, while activating metabolic systems of the body in general [9, 30]. It was reported that hypermetabolic condition in hyperthyroidism was associated with an increase in free radical formation and lipid peroxidation levels [9, 10, 31]. In previous studies, there are conflicting results about oxidative stress in hyperthyroidism. In some studies, it was demonstrated that the products of lipid peroxidation were decreased [30, 32]. On the contrary, Fernandez et al. [9] and Dumitriu et al. [33] found high products of lipid peroxidation. Similarly, Iangalenko et al. [34] found that lipid peroxidation was increased in hyperthyroid patients. Asayama et al. [17] showed that the damaging effect of lipid peroxidation was increased diminishing antioxidant enzymes in experimental hyperthyroidism.

The effects of various antioxidants in serum are additive and the cooperation of antioxidants in human serum provides protection of the organism against attacks by free radicals [25]. Thus, measurement of individual antioxidants may not accurately reflect the true antioxidant status of the organism. Thus, measurement of TAC should be essential in evaluating the true state of antioxidant status [22, 23, 35].

The most widely used methods for oxidative status measurement are colorimetric, or involve either fluorescence or chemiluminescence [36–38]. The fluorescence and chemiluminescence methods require sophisticated techniques, and these improved systems are not present in many routine clinical biochemistry laboratories. On the other hand, even when these technologies are available, their routine usage is limited [22].

There are several limitations in our study. First, this study is the cross-sectional study design. Second, the sample size was not large, but on the other hand, our results were strongly significant, the study groups were quite homogenous. However, a large sample would have increased the power to detect oxidative status in patients with hyperthyroidism.

Our data illustrates that oxidants are increased and antioxidants are decreased in patients with hyperthyroidism; as a result, the oxidative–antioxidative balance is shifted to the oxidative side. Increased oxidative stress may play a role in the pathogenesis of hyperthyroidism. It is believed that supplementation of antioxidants vitamins such as vitamins C and E may be helpful for these patients. The automated assay is a reliable and easily applicable method for serum TAC measurement during the course of hyperthyroidism. Further studies including larger number of patients are needed to clarify the results in with hyperthyroidism.

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Conflict of interest None.

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