The evaluation of selected oxidative stress parameters in patients with hyperthyroidism

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Abstract: Introduction. Hyperthyroidism induces the acceleration of the basic metabolism and increases cellular oxygen utilization, consequently intensifies reactive oxygen species production and disturbs the oxidant-antioxidant balance. **Objectives.** The objective of this study was to evaluate the selected oxidative stress parameters in patients with hyperthyroidism by analysis of the reactive oxygen species neutralizing enzymes activity – superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase (CAT), the estimation of free radical processes intensity – concentration of malondialdehyde (MDA), sulfhydryl groups (SH) in proteins and by quantification of the serum total antioxidant status (TAS). **Patients and methods.** Twenty-seven patients treated for hyperthyroidism and 12 healthy individuals were enrolled in the study. Enzyme activity (SOD, GSHPx, CAT), MDA and concentration of SH groups were analysed in erythrocytes, while TAS was measured in serum. **Results.** Patients with hyperthyroidism compared with healthy subjects were characterized by a higher GSHPx activity in erythrocytes, lower serum TAS, the lower content of SH groups in proteins and the lower MDA concentration in erythrocytes. **Conclusions.** Our results suggest that hyperthyroidism increases oxidative stress and disturbs oxidant-antioxidant balance in the body. Thyreostatic treatment, if it does not lead total metabolic correction, can only reduce oxidant-antioxidant disorder, but it does not eliminate it entirely.

Key words: hyperthyroidism, oxidant-antioxidant balance, oxidative stress

INTRODUCTION

Numerous clinical studies and experiments on animal models demonstrate that oxidative stress is an important element of pathogenesis of multiple pathological changes and the physiological process of tissue ageing [1,2]. Oxidative stress, according to Sies, is an excessive shift of the oxidant-antioxidant balance towards the oxidation reaction [3]. Bartosz highlights the role of increased levels of reactive oxygen species (ROS) as the main factor responsible for oxidation of molecules [1]. The natural source of reactive oxygen species in the human organism is the mitochondrial respiratory chain. About 1–4% of oxygen used by mitochondria undergo a one-electron reaction due to the "electron leak from the respiratory chain" phenomenon, forming a superoxide radical. Hormones of the thyroid gland exert a significant regulatory effect on tissue oxidation processes. If their level is too high it results in the accelerated basal metabolism and increased oxygen consumption by cells [4]. The consequence of hyperthyroidism is the enhanced endogenous synthesis of ROS leading to oxidative stress [4,5].

The aim of this study was to determine the influence of hyperthyroidism on the parameters of oxidative stress by analysis of activity of antioxidant enzymes in erythrocytes: superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase (CAT); analysis of indices illustrating the intensity of free radical processes in the organism: malonyldialdehyde (MDA) level; and evaluation of the content of thiol groups (SH) in proteins and plasma total antioxidant status (TAS).

PATIENTS AND METHODS

The study included 39 persons (25 women and 14 men) at the ages of 27–78 years. Twenty-seven persons from this group had confirmed hyperthyroidism (Graves-Basedow disease, toxic nodular goitre, single autonomic nodule). All patients from this group were qualified for radioiodine treatment and in the period preceding the radioiodine therapy they were treated with thiamazole at a dose of 5 mg daily. Thiamazole was discontinued three days before administration of radioiodine.

The control group consisted of 12 healthy persons, in whom the serum levels of thyroid-stimulating hormone (TSH) were within normal limits.

Persons with severe heart failure, diabetes, acute coronary event within the preceding year, neoplastic disease, hepatic

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insufficiency, renal insufficiency, diseases requiring anti-inflammatory treatment (non-steroidal anti-inflammatory drugs, glycocorticosteroids) and numerous diseases requiring polypharmacotherapy were excluded from the study. Characteristics of the study and control group are presented in Table 1.

From each person participating in the study, 4 ml of venous blood were collected to determine the following parameters:

1) SOD activity (EC 1.15.1.1.) in erythrocytes by the Misra and Fridovich method [6]
2) EC activity (1.11.1.6.) in erythrocytes by the Beers and Sizer method [7]
3) GSHPx activity (EC 1.11.1.9.) in erythrocytes by the Little and O'Brien method [8]
4) MDA level in erythrocytes by the Placer et alli method [9]
5) level of SH groups of proteins with the Ellman reagent according to Rice-Evans et al. [10]
6) TAS according to Benzie and Strain [11] in modification by Bartosz [1].

Results were subjected to statistical analysis, with calculation of the arithmetic mean, standard deviation and the median. By the Kolmogorov-Smirnov test the distribution of the values was tested for consistence with the normal distribution. Statistical significance was evaluated with the Mann-Whitney test when the distribution of values did not meet the conditions for a normal distribution.

Consent No. RNN/42/04/KB for execution of this medical experiment was obtained from the Bioethical Committee.

Table 1. Characteristics of investigated groups

	Control group n = 12	Study group n = 27
Age (years)	47.70 ±15.4	56.90 ±14.1
TSH (norm: 0.3–5.0 µIU/ml)	1.14 ±0.79	0.24 ±0.32*
FT_3 (norm: 2.3–6.0 pg/ml)	–	3.79 ±1.29
FT_4 (norm: 8.5–17 pg/ml)	–	18.01 ±4.24
Graves-Basedow disease	–	13
Toxic nodular goitre	–	8
Single autonomic nodule	–	6
Arterial hypertension	2	8
Ischaemic heart disease	2	3
Atrial fibrillation	1	3
NYHA I-II heart failure	1	–

* $p < 0.005$

FT_3 – free triiodothyronine, FT_4 – free thyroxine, TSH – thyroid stimulating hormone

RESULTS

In patients with hyperthyroidism, despite treatment with thyrostatic (thiamazole) resulting in obtaining thyroid hormone levels and the TSH approximating normal values, a significant oxidative-reduction imbalance was observed in comparison with healthy persons. Patients with hyperthyroidism, in comparison to healthy individuals, demonstrated a greater GSHPx activity in erythrocytes (11.21 ±10.20 vs. 1.61 ±1.55 U/g of haemoglobin; $p < 0.001$), a decreased content of SH groups in proteins (0.88 ±0.14 vs. 1.04 ±0.14 nmol/mg; $p < 0.05$) and a lower TAS (1.09 ±0.77 vs. 2.14 ±1.58; $p < 0.05$). The level of MDA in patients with hyperthyroidism was 0.138 ±0.040 µmol/g Hgb, whereas in the control group it was 0.190 ±0.045 µmol/g Hgb ($p < 0.05$). All listed differences in the parameters of oxidative stress were statistically significant. Differences in the SOD and CAT activity in erythrocytes between the control and study groups failed to reach the statistical significance (Tab. 2).

DISCUSSION

The status of oxidative-reduction balance depends on numerous factors. On one hand, on the destructive influence of reactive oxygen species, like the superoxide anion radical, hydrogen peroxide or hydroxyl radical and other oxidative factors. On the other hand, on the effectiveness of a number of mechanisms protecting the organisms from effects of free radicals. There is an enzymatic system that catalyses neutralisation of free radicals (SOD, CAT, GSHPx). In addition, there are several compounds with antioxidant properties. Depending on their character, we distinguish: hydrophilic (protecting the aqueous environment of the organism), i.e. ascorbates, uric acid, glutathione, albumins, creatinine, and hydrophobic (protecting protein-lipid membranes), i.e. tocopherols, carotenoids , bilirubin, oestradiol derivatives. They can inactivate free radicals, bind fatty acids and metal ions, or react themselves with the radicals leading to the formation of nontoxic products. The main source of ROS in the organism is the mitochondrial respiratory chain, where via a cycle of reactions an oxygen molecule undergoes a four-electron reduction with the production of a portion of energy stored as the ATP. Several percent of oxygen molecules undergo a one-electron reduction that results in the formation of a superoxide anion radical.

Thyroid gland hormones exhibit a multifaceted effect on the human organism. Among others, they influence the basal metabolic rate; the overproduction of thyroid gland hormones results in an increased rate of metabolism and oxygen consumption by cells. These processes are reflected by an elevated number of mitochondria in cells, an increased size of mitochondria and the number of cristae, as well as degenerative changes in mitochondria observed in persons with hyperthyroidism [4,12,13]. During thyrotoxicosis, researchers observed either elevated level of reactive oxygen species in the body: superoxide anion radical [14] and hydrogen peroxide [15], or an increased activity of enzymes taking part in the escalation of free radical processes, e.g. xanthine oxidase [16] or the NADPH oxidase [17]. Numerous studies focused on the evaluation of indirect indicators of oxidative-reduction balance

Table 2. Comparison of selected oxidative stress parameters in patients with hyperthyroidism and in healthy persons

Parameter	Unit	Control group n = 12			Study group n = 27			p
		mean	standard deviation	median	mean	standard deviation	median	
Superoxide dysmutase (SOD)	U/g Hgb	2819	651	2954	3139	583	3107	0.45*
Glutathione peroxidase (GSHPx)	U/g Hgb	1.61	1.55	0.88	11.21	10.20	8.38	<0.001**
Catalase (CAT)	U/g Hgb	20.59	5.31	19.42	18.17	4.21	18.53	0.77*
Malonyldialdehyde (MDA)	µmol/g Hgb	0.190	0.045	0.198	0.138	0.040	0.14	<0.05*
Thiol groups in proteins (SH)	nmol/mg	1.04	0.14	1.06	0.88	0.14	0.89	<0.05*
Plasma total antioxidant status (TAS)	1-electrode Trolox equivalents	2.14	1.58	1.38	1.09	0.77	0.85	<0.05**

* Statistical significance was assessed with the Kolmogorov-Smirnov test.

** Statistical significance was assessed with the Mann-Whitney U test.

Hgb – haemoglobin

in hyperthyroidism, like levels of lipid peroxidation products and antioxidant compounds, and the activity of enzymes neutralising the ROS or the TAS. Some of the listed parameters were also evaluated by the authors of the present study.

The main line of the organism defence against harmful effects of reactive oxygen species include: SOD, GSHPx and CAT. The activity of SOD in erythrocytes of patients treated for hyperthyroidism was 11% higher than in the healthy population, however this difference did not reach statistical significance (Fig.). In contrast, the GSHPx activity in erythrocytes of persons with hyperthyroidism was significantly higher (11.21 U/g of haemoglobin vs. 1.61 U/g of haemoglobin, $p < 0.001$). On the other hand, no significant difference was observed in the CAT activity between the investigated groups. Available data show that the results of research on antioxidant enzymes activity in hyperthyroidism are contradictory. Some investigators evaluating the SOD activity in erythrocytes of persons with hyperthyroidism recorded its decreased activity [18,19]. The other observed an increased activity of SOD [20,21].

The activity of GSHPx in patients with hyperthyroidism was assessed by Komosinska-Vassev et al. [20] who observed that it was elevated, whereas other researchers demonstrated a reduced activity of this enzyme in similar groups [18,22,23]. Likewise, the elevated CAT activity in patients with hyperthyroidism was shown in some studies [20,23], and the decreased in other papers [24] in comparison with a population of healthy individuals. Authors of the studies where patients were treated with thyrostatics emphasised that after euthyroidism had been obtained, the activity of the investigated enzymes reached the level comparable with the activity observed in comparative groups, regardless of whether the baseline activities of these enzymes were increased or decreased [20,22,23].

Another element of the antioxidant barrier in the organisms are low-molecular-weight compounds. The level of these compounds can be determined separately (ascorbates, tocopherols, glutathione), or without distinguishing components of the barrier – as the TAS. The main elements determining the antioxidant plasma activity include: serum uric acid level and the content of SH groups in plasma proteins [1]. In the study conducted by the authors of the present paper, the TAS in persons with hyperthyroidism was lower by 49% than in the control group. Other investigators observed a similar reduction in the plasma total antioxidant status in hyperthyroidism [18,20].

Parameters commonly evaluated in studies illustrating the intensity of oxidative stress in the body include lipid peroxidation products. By oxidising lipids, mainly cell membrane phospholipids, reactive oxygen species lead to the loss of their physiological function, and, in addition, to the formation of numerous lipid peroxides and their metabolites, toxic for the body, which themselves are able to cause damage to proteins and the DNA [1]. One of the compounds originating in the process of lipid peroxidation is the MDA. It is believed that there is a strong correlation between the level of thyroid hormones and the intensity of lipid peroxidation, both in humans and in animals [25]. It is supported by observed in persons with hyperthyroidism high levels of the MDA [20-22,26]. Our own studies have not confirmed this correlation; the MDA level in patients with hyperthyroidism was significantly lower, by 37%, than in healthy persons (0.190 µmol/g of haemoglobin vs. 0.138 µmol/g of haemoglobin, $p < 0.05$). It should be however emphasised that as a result of the thyrostatic therapy the level of thyroid hormones only slightly exceeded the normal

range. Moreover, this observation is not isolated. Mano et al. [27] demonstrated a reduced level of lipid peroxidation products in animals with iatrogenic hyperthyroidism.

The last parameter to be evaluated was the thiol groups content in plasma proteins, which are particularly sensitive to effects of free radicals. The groups of SH in consequence of oxidation form disulphide bridges, which can alter the structure and physiological function of proteins. Plasma proteins containing SH groups have a protective function for other, "more valuable" molecules, because SH groups are easily mended in the reduction process, and proteins highly damaged by free radicals are subject to proteolytic degradation without any harm to the organism. A reduction in the content of thiol groups in plasma proteins illustrates the intensity of free radical processes [1]. Such results have been obtained in this study. The group of patients with hyperthyroidism was characterised by a fifteen-percent lower content of SH groups in proteins in comparison to euthyroid persons (1.04 nmol/mg vs. 0.88 nmol/mg, $p < 0.05$). A similar observation was made by Ademoglu et al. [28], who recorded a thirty-seven-percent reduction in the SH content in serum of individuals with Graves-Basedow disease, with reference to the control group.

Recapitulating, the obtained results indicate that:

1) hyperthyroidism enhances oxidative stress leading to oxidative-reduction imbalance in the body, as reflected by an increased activity of glutathione peroxidase, a reduced plasma total antioxidant status and a reduction in the content of thiol groups in plasma proteins
2) treatment with thyrostatics, if it does not lead to total metabolic correction, can alleviate, but does not eliminate the oxidative-reduction imbalance produced by hyperthyroidism.

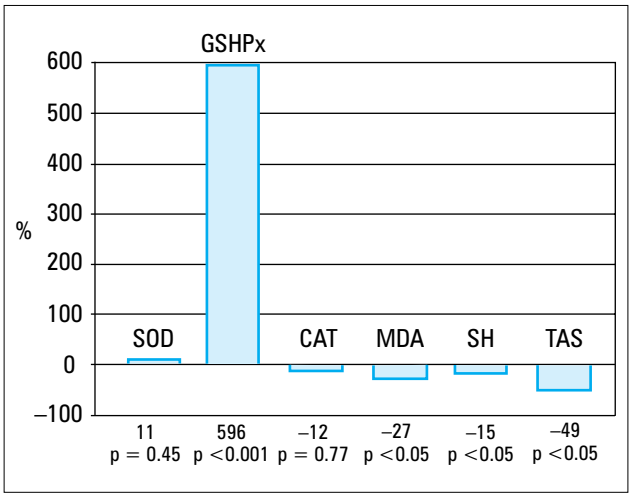

Fig. Percentage differences of the tested parameters in patients with hyperthyroidism with reference to the control group. CAT – catalase, GSHPx – glutathione peroxidase, MDA – malonyldialdehyde, SH – thiol groups in protein, SOD – superoxide dysmutase, TAS – plasma total antioxidant status

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