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Wskaźniki stresu oksydacyjnego w przebiegu nadczynności tarczycy

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Streszczenie

Wstęp: Wcześniejsze badania wskazują na występowanie stresu oksydacyjnego w stanach nadczynności tarczycy. Celem niniejszej pracy była ocena wpływu nadczynności tarczycy na procesy peroksydacji lipidów, oksydację lipoprotein osocza oraz stan antyoksydacyjny organizmu. Oceniano przydatność oznaczanych parametrów biochemicznych jako wskaźników stresu oksydacyjnego w hipertyreozie.

Materiał i metody: Badaniami objęto 25 pacjentów z jawną nadczynnością tarczycy w przebiegu choroby Gravesa-Basedowa lub wola guzkowego nadczynnego oraz 20 zdrowych ochotników stanowiących grupę kontrolną. Nasilenie procesów peroksydacji lipidów oceniano poprzez pomiar stężenia nadtlenków lipidów oraz pomiar stężenia dialdehydu malonowego oznaczanego łącznie z 4-hydroksynonenalem w surowicy (MDA + 4-HNE). Wskaźnikiem oksydacji lipoprotein było stężenie przeciwciał przeciwko oksydowanym LDL (anti-oxLDL, *autoantibodies against oxidised LDL*). Zmiany w systemie obrony antyoksydacyjnej organizmu oceniano poprzez pomiar całkowitego stanu antyoksydacyjnego surowicy (TAS, *total antioxidant status in serum*) oraz aktywność dysmutazy ponadtlenkowej erytrocytów (SOD, *superoxide dismutase activity*).

Wyniki: W badanej grupie pacjentów z nadczynnością tarczycy obserwowano zwiększone stężenie produktów peroksydacji lipidów w surowicy, zarówno nadtlenków, jak i MDA + 4-HNE. Nie stwierdzono istotnych statystycznie różnic między grupą badaną a kontrolną w stężeniu przeciwciał przeciwko oksydowanym LDL oraz w wartościach wskaźników obrony antyoksydacyjnej organizmu (TAS, SOD).

Wnioski: Wyniki badań wskazują na nasilenie przemian oksydacyjnych wywołanych działaniem wysokich stężeń hormonów tarczycy, któremu nie towarzyszy odpowiedź systemu antyoksydacyjnego organizmu. Podwyższone stężenie produktów peroksydacji lipidów w surowicy, zarówno nadtlenków, jak i dialdehydu malonowego oraz 4-hydroksynonenalu, może być użytecznym wskaźnikiem stresu oksydacyjnego w przebiegu nadczynności tarczycy.

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***Słowa kluczowe**: nadczynność tarczycy, stres oksydacyjny, peroksydacja lipidów, oksydacja LDL, autoprzeciwciała*

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Oxidative stress markers during a course of hyperthyroidism

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Abstract

Introduction: Previous studies have shown the presence of oxidative stress in hyperthyroid patients. The aim of this study was to evaluate the influence of hyperthyroidism on lipid peroxidation, plasma lipoprotein oxidation and antioxidant status. We have estimated the clinical utility of the biochemical parameters analysed as markers of oxidative stress in hyperthyroidism.

Material and methods: Twenty five patients with overt hyperthyroidism because of Graves' disease or toxic multinodular goitre and 20 healthy subjects were included in the study. Lipid peroxidation was evaluated by measurement of peroxides and malondialdehyde with 4-hydroxynonenal (MDA + 4-HNE) concentrations. Autoantibodies against oxidised LDL (anti-oxLDL) were assayed as a marker of lipoprotein oxidation. Changes in the antioxidant defence system were estimated by measurement of total antioxidant status in serum (TAS) and erythrocyte superoxide dismutase activity (SOD).

Results: A significant increase in serum concentration of peroxides and MDA + 4-HNE was observed in patients with hyperthyroidism. However, no difference was found in anti-oxLDL concentration and antioxidant status parameters (TAS, SOD) between the control group and the patient group.

Conclusions: Our results indicate an intensification of the oxidative processes caused by an excess of thyroid hormones, which is not accompanied by a response from the antioxidant system. Elevated concentrations of lipid peroxidation products in serum, both peroxides and malondialdehyde with 4-hydroxynonenal, may be useful as markers of oxidative stress during the course of hyperthyroidism.

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***Key words**: hyperthyroidism, oxidative stress, lipid peroxidation, LDL oxidation, autoantibodies*

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Introduction

Oxidative stress accompanying hyperthyroidism is caused by increased synthesis of reactive oxygen species and changes in the antioxidant defence system. An elevated metabolic rate and acceleration of oxygen consumption in hyperthyroidism leads to increased generation of reactive oxygen species. The antioxidant defence system includes both enzymatic and non-enzymatic components. The antioxidant enzymes comprise superoxide dismutase, glutathione peroxidase and catalase. Non-enzymatic small molecules that act as antioxidants include vitamins E and C, glutathione and coenzyme Q [1–4]. Both stimulation and inhibition of antioxidant enzyme activity were observed in hyperthyroidism [1, 2, 4]. Changes in concentration of non-enzymatic components were also noted in hyperthyroidism [3, 5].

An enhanced generation of reactive oxygen species concomitant with inactivation of the antioxidant systems leads to intensified cellular lipid peroxidation [1]. Hyperthyroidism is associated with an increase in lipid peroxidation product concentration in the blood of patients. Lipid peroxidation may be estimated by measurement of primary products (conjugated dienes, peroxides) as well as the final products of this process (malondialdehyde, 4-hydroxynonenal) [1, 6].

Intensification of lipid peroxidation in patients with hyperthyroidism may be accompanied by an increase in oxidative modification of plasma lipoproteins [7–9]. Reactive oxygen species generated in artery walls participate in a low-density lipoprotein oxidation process. These lipoproteins display immunogenic properties and therefore autoantibodies against oxidised LDL produced by the immune system may be used as markers of lipoprotein oxidation [10].

The aim of our study was to evaluate the effect of hyperthyroidism on the lipid peroxidation process and plasma lipoprotein oxidation and to estimate antioxidant status. We evaluated the utility of some biochemical parameters as oxidative stress markers in hyperthyroidism.

Material and methods

Twenty five patients (21 women and 4 men) with overt hyperthyroidism were investigated. Eight patients were diagnosed with Graves' disease and 17 with toxic multinodular goitre. Diagnosis of hyperthyroidism was based on clinical examination and was confirmed by measurement of thyroid stimulating hormone (TSH) and thyroid hormone levels. The age of the patients ranged from 27 to 76. The control group included 20 healthy subjects (18 women and 2 men) of 26 — 68 years with normal TSH concentration.

Heparinised blood was used to determine SOD activity in erythrocytes. The remaining parameters were assayed in serum. Serum and erythrocyte lysates were stored at –70°C until assayed. Malondialdehyde concentration was assayed in combination with 4-hydroxynonenal using a commercially available kit, Bioxytech LPO-586 (Oxis, USA). Peroxide concentration was measured with OxyStat, and autoantibodies against oxidised LDL — oLAB by ELISA (Biomedica, Austria). Total antioxidant status and superoxide dismutase activity were assayed by TAS and Ransod kits (Randox Laboratories, UK). Total cholesterol and triglyceride concentrations were measured by the enzymatic method and HDL cholesterol concentration by a precipitation method. LDL cholesterol concentration was calculated by Friedewald's formula. TSH, FT_4 and FT_3 were measured by electrochemiluminescence immunoassay (Elecsys, Roche) in the Department of Laboratory Medicine. FT_3 concentration was determined only in the group of patients with hyperthyroidism. Results were expressed as median and range. The Mann-Whitney U test was used to determine whether differences between the control group and patient group were significant, with $p < 0.05$ taken as statistically significant.

Results

The values of the laboratory parameters evaluated in hyperthyroid patients and in the control group are presented in Tables I and II. For each parameter median value and the range from minimum to maximum are provided.

Parameters describing thyroid function and serum lipid profile are reported in Table I. Serum concentrations of TSH and FT_4 in the control group were within the range of reference values. Statistical analysis revealed that patients with hyperthyroidism were characterised by significantly decreased total cholesterol ($p < 0.01$), LDL cholesterol ($p < 0.05$) and HDL cholesterol ($p < 0.01$)

Table I
Thyroid function parameters and serum lipid profile in hyperthyroid patients (n = 25) and control subjects (n = 20)

Tabela I
Parametry czynności tarczycy i profil lipidowy surowicy u chorych na nadczynność tarczycy (n = 25) i u osób z grupy kontrolnej (n = 20)

Laboratory parameters (reference values)	Hyperthyroid patients Median (min–max)	Control group Median (min–max)
TSH [mIU/l] (0.3500–4.9400)	0.0012*** (0.0001–0.0840)	1.2790 (0.4064–2.5440)
FT_4 [ng/dl] (0.70–1.48)	1.94*** (0.74–15.00)	0.940 (0.80–1.16)
FT_3 [pg/ml] (1.71–3.71)	5.50 (3.76–30.00)	
Total cholesterol [mg/dl] (< 200)	171** (110–235)	207 (135–289)
LDL cholesterol [mg/dl] (< 130)	112* (50–174)	130 (55–226)
HDL cholesterol [mg/dl] (> 35)	49** (25–77)	62 (45–98)
Triglycerides [mg/dl] (< 150)	93* (45–205)	74 (32–155)

Statistical significance versus the control group; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table II
Parameters of lipid peroxidation, LDL oxidation and antioxidant system in hyperthyroid patients (n = 25) and control subjects (n = 20)

Table II
Parametry peroksydacji lipidów, oksydacji LDL oraz układu antyoksydacyjnego u chorych na nadczynność tarczycy (n = 25) oraz u osób z grupy kontrolnej (n = 20)

Laboratory parameters	Hyperthyroid patients Median (min–max)	Control group Median (min–max)
Peroxides [μmol/l]	340 *** (34–1037)	47 (30–520)
MDA + 4-HNE [μmol/l]	1.52** (0.40–2.80)	0.97 (0.40–2.02)
anti-oxLDL [mU/ml]	332 (161–2457)	450 (138–2099)
TAS [mmol/l]	1.53 (1.17–1.83)	1.49 (1.33–1.88)
SOD [U/g Hb]	1197 (800–2158)	1086 (738–1935)

Statistical significance versus the control group; **$p < 0.01$; ***$p < 0.001$

values but elevated triglycerides ($p < 0.05$) compared to the control group.

Lipid peroxidation indices, lipoprotein oxidation marker and values of antioxidant system parameters are shown in Table II. We found a significant elevation of lipid peroxidation products, both peroxides ($p < 0.001$) and MDA + 4-HNE ($p < 0.01$), in patients with hyperthyroidism. No significant difference was observed between patients and the control group in the concentration of autoantibodies against oxidised LDL (anti-oxLDL). TAS and erythrocyte SOD activity did not change in patients with hyperthyroidism.

Discussion

The present study demonstrates an intensification of the lipid peroxidation process in hyperthyroidism. Elevated peroxide as well as malondialdehyde and 4-hydroxynonenal concentrations were found in the serum of patients with hyperthyroidism. Our results are in agreement with the data of others [1–5]. According to Komosinska-Vassev et al. [1], Ademoglu et al. [5] and Seven et al. [11], increased lipid peroxidation is associated with an excess of thyroid hormones, while inhibition of peroxidation takes place in the euthyroid state after antithyroid treatment.

Elevated lipid peroxidation may be accompanied by intensified plasma lipoprotein oxidation. Elevated LDL oxidation in patients with hyperthyroidism was noted by Costantini et al. [7] and Sundaram et al. [8]. Both authors used measurement of lipid peroxidation product concentration in isolated LDL. Dirican and Tas [9] have also shown increased LDL susceptibility to oxidation in experimental hyperthyroidism.

In our study autoantibodies against oxidised LDL were assayed as a lipoprotein oxidation marker, although their concentration did not differ significantly between the examined group and the control group. No significant changes in concentration of anti-ox LDL in hyperthyroidism were reported by Resch et al. [12] either. Autoantibodies against oxidised LDL concentration have been used as a marker of LDL oxidative modification by numerous authors, mainly in patients with coronary artery disease [13, 14]. However, other studies have shown that anti-oxLDL concentration is in an inverse relationship with plasma oxLDL concentration in healthy subjects [15] and that synthesis of these autoantibodies depends on age [16]. This parameter may also be a marker of total antibody production in the organism, especially in autoimmune disease [16]. Therefore serum anti-oxLDL concentration does not seem to be a good marker of oxidative stress in hyperthyroidism.

We encountered a number of problems in evaluating LDL oxidation in hyperthyroidism because many different factors have an influence on this process. The increased generation of reactive oxygen species in hyperthyroidism may contribute to intensified LDL oxidative modification but, at the same time, a decreased concentration of these lipoproteins in serum, caused by intensified catabolism, may reduce their oxidation effect. A decrease in LDL lipoprotein level in hyperthyroidism is manifested by low cholesterol concentration in serum. Significantly decreased total cholesterol and LDL cholesterol concentrations were noted in the

patient group. The factor that might directly inhibit oxidative modification of low density lipoprotein is thyroxine. Examination in vitro revealed that thyronine analogue L-T_4 in the physiological concentration inhibited LDL oxidation [17].

Lipid peroxidation intensification may also be caused by a reduced antioxidative defence of the organism. In our study changes in total antioxidant status were not noted in patients with hyperthyroidism in comparison with the control group. Komosinska-Vassev et al. [1] noted a decrease in TAS values in the serum of patients with Graves' disease and its elevation after antithyroid treatment. Resch et al. [12] noted a decrease in non-enzymatic antioxidant concentration and enzymatic activity intensification in hyperthyroidism. In our examination no significant changes in SOD activity were noted in patients with hyperthyroidism. The superoxide dismutase activity is characterised by non-specific changes in the course of hyperthyroidism. Both an elevation [1, 11, 18] and a decrease [19] in erythrocyte SOD activity were reported in patients with hyperthyroidism.

These differences in antioxidant enzyme activity may be caused by various mechanisms. The reactive oxygen species contribute to an intensified synthesis of antioxidant enzymes in tissues and hence their elevated activity may be a manifestation of adaptation mechanisms in response to oxidative stress. A decreased activity of antioxidant enzymes or a decreased non-enzymatic antioxidant concentration may be caused by their intensified utilisation in protection against oxidative damage to tissues [1, 18]. There are a number of factors that may influence antioxidant system activity. In experimental examination on animals, antioxidant enzyme activity was affected by the age of animals with induced hyperthyroidism [20].

In our examination no adaptive changes in the antioxidant defence system of the organism were noted in patients with hyperthyroidism. Therefore determination of antioxidant enzyme activity and non-enzymatic antioxidant concentration seems to be less useful in the evaluation of oxidative stress in hyperthyroidism.

Conclusions

Our results indicate intensification of the oxidative processes caused by thyroid hormone excess, which are not accompanied by an adaptive response of the antioxidant system. An augmented concentration of lipid peroxidation products in serum, both peroxides and malondialdehyde with 4-hydroxynonenal, may constitute a useful marker of oxidative stress in the course of hyperthyroidism.

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