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MARKERS OF LIPID AND PROTEIN OXIDATION IN THE BLOOD OF WOMEN AND MEN WITH AUTOIMMUNE HASHIMOTO'S THYROIDITIS

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МАРКЕРИ ОКИСНЕННЯ ЛІПІДІВ І БІЛКІВ У КРОВІ ЖІНОК І ЧОЛОВІКІВ З АУТОІМУННИМ ТИРЕОЇДИТОМ ХАШИМОТО

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ABSTRACT

Purpose: The aim of this study was to analyze changes in markers of oxidative stress (lipid peroxidation and oxidative modification of proteins) and the total antioxidant capacity in the blood of women and men with autoimmune Hashimoto's thyroiditis (HT).

Methodology. This study was carried out in a group of 153 individuals. The group of women participating in the study consisted of 109 individuals (71.24 %), while the group of men consisted of 44 individuals (28.76 %). All persons were divided into two groups: 1) euthyroidism (n = 64; men – n = 18, and women – n = 46); and 2) Hashimoto's autoimmune thyroiditis with subclinical hypothyroidism (n = 89; men – n = 26, and women – n = 63). The functioning of the thyroid gland was additionally verified by measuring the concentration of thyrotropin (TSH), free triiodothyronine (fT3), free tetraiodothyronine (thyroxine, fT4), and antibodies against thyroid peroxidase (anti-TPO). The concentration of thyrotropin, triiodothyronine, free thyroxine and the concentration of antibodies against thyroperoxidase in human serum were determined using the electrochemiluminescence method "ECLIA" on the immunological analyzer Elecsys Cobas e 411 (Hitachi, Japan). In each group of women and men with euthyroidism and Hashimoto's autoimmune thyroiditis with subclinical hypothyroidism, the 2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives of protein oxidative modification (OMP), and total antioxidant capacity (TAC) were determined.

Scientific novelty. Statistically significant changes in levels of oxidative stress markers were not observed. In women with HT, elevated TBARS levels with simultaneously increased TAC levels in the plasma and erythrocytes were observed. Additionally, levels of aldehydic and ketonic derivatives of oxidative modification of proteins in the blood of women with HT were lower compared to the women with the euthyroid state. In men with HT, levels of markers of oxidative stress (except TAC levels in the plasma) were lower compared to those obtained in men with the euthyroid state.

Conclusions. Hashimoto's thyroiditis with subclinical hypothyroidism does not have a direct influence on levels of biomarkers of lipid and protein oxidation. The results obtained in the current study highlight the need for future investigations of biomarkers of lipid and protein oxidation, especially depending on the duration of this disease.

Keywords: autoimmune thyroiditis; oxidative stress; subclinical hypothyroidism; 2-thiobarbituric acid reactive substances (TBARS); carbonyl derivatives of protein oxidative modification (OMP); total antioxidant capacity (TAC)

АНОТАЦІЯ

Мета: Метою даного дослідження був аналіз змін маркерів оксидативного стресу (перекисного окиснення ліпідів та окиснювальної модифікації білків), а також загальної антиоксидантної активності в крові жінок і чоловіків з аутоімунним тиреоїдитом Хашимото (АІТ).

Методологія. Це дослідження проводилося в групі 153 осіб. Група жінок, які брали участь у дослідженні, складалася з 109 осіб (71,24 %), а група чоловіків – з 44 осіб (28,76 %). Усіх осіб було розділено на дві групи: 1) еутиреоз (n = 64; чоловіки – n = 18, жінки – n = 46); 2) аутоімунний тиреоїдит Хашимото з субклінічним гіпотиреозом (n = 89; чоловіки – n = 26, жінки – n = 63). Функціонування щитовидної залози додатково перевіряли шляхом вимірювання концентрації тиреотропіну (ТТГ), вільного трийодтироніну (fT3), вільного тетрайодтироніну (тироксину, fT4), антитіл до тиреопероксидази (анти-ТПО). Концентрацію тиреотропіну, трийодтироніну, вільного тироксину та концентрацію антитіл проти тиреопероксидази в сироватці крові визначали електрохемілюмінесцентним методом «ECLIA» на імунологічному аналізаторі Elecsys Cobas e 411 (Hitachi, Японія). У кожній групі жінок і чоловіків з еутиреозом і аутоімунним тиреоїдитом Хашимото з

субклінічним гіпотиреозом визначали у крові вміст ТБК-активних продуктів (TBARS), карбонільних похідних окиснювальної модифікації білків (ОМБ), а також загальну антиоксидантну активність (ТАС).

Наукова новизна. Статистично значущих змін рівнів маркерів окиснювального стресу не спостерігалося. У жінок з АІТ спостерігали підвищення рівня TBARS з одночасним підвищенням рівня ТАС у плазмі та еритроцитах. Крім того, рівні альдегідних і кетонових похідних окиснювальної модифікації білків у крові жінок з АІТ були нижчими порівняно з результатами, отриманими в групі жінок з еутиреозом. У чоловіків із АІТ рівні маркерів оксидативного стресу (крім рівня ТАС у плазмі) були нижчими порівняно з такими у чоловіків із еутиреозом.

Висновки. Аутоімунний тиреоїдит Хашимото з субклінічним гіпотиреозом у наших дослідженнях не має прямого впливу на рівні біомаркерів окиснення ліпідів і білків. Результати, отримані в поточному дослідженні, підкреслюють необхідність майбутніх досліджень біомаркерів окиснення ліпідів і білків, особливо в залежності від тривалості цього захворювання.

Ключові слова: аутоімунний тиреоїдит; окиснювальний стрес; субклінічний гіпотиреоз; ТБК-активні продукти (TBARS); карбонільні похідні окиснювальної модифікації білків (ОМБ); загальна антиоксидантна активність (ТАС)

Introduction

Autoimmune thyroiditis is a chronic inflammatory disease that is associated with the destruction and damage of thyroid follicles and cells. At the same time, the aggression of the immune system is directed against the thyroid gland (Ralli et al., 2020). AITD development occurs due to loss of immune tolerance and reactivity to thyroid autoantigens: thyroid peroxidase (TPO), thyroglobulin (TG), and thyroid stimulating hormone receptor (TSHR). This leads to infiltration of the gland by T cells and B cells that produce antibodies specific for clinical manifestations of disease (Antonelli et al., 2015; Mikoś et al., 2014, Kristensen 2016). T cells in Hashimoto's thyroiditis (HT) induce apoptosis in thyroid follicular cells, leading ultimately to the destruction of the gland. Cytokines (IL-1α, IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, IL-14, TNF-α and IFN-γ) are involved in the pathogenesis of thyroid diseases working in both the immune system and directly targeting the thyroid follicular cells. They are involved in the induction and effector phase of the immune response and inflammation, playing a key role in the pathogenesis of autoimmune thyroid disease (Mikoś et al., 2014; Khan et al., 2015).

Its prevalence is about 1 in 1,000 people and increases with age, affecting up to 40% of elderly women (Hollowell et al., 2002). Thyroid failure is seen in up to 10% of the population. The incidence of HT is higher in countries where there is excess iodine in the diet, approximately 1.3%, compared to 1% in countries that are iodine-sufficient (Zimmermann and Boelaert, 2015). It affects more women than men with a female-to-male ratio of 18:1. This is due to the production of hormones and the fact that males are protected from autoimmune diseases by lymphoid cells of the innate immune system (Rocchi et al., 2008). There is a peak frequency during the fourth decade, and the mean age of presentation is 35 years (Rocchi et al., 2008). Autoimmune thyroiditis can be accompanied by hypothyroidism (a decrease in the ability of the gland to produce hormones), hyperthyroidism, euthyroidism, and diffuse and nodular changes. In addition, with the disease, the appearance of cysts and nodes is not uncommon (Franco et al., 2013).

Recently, scientific data have appeared indicating a significant role of oxidative stress in the development of autoimmune thyroid disorders (Rybakova et al., 2020; Kochman et al., 2021). It is assumed that the synthesis of thyroid hormones depends on the concentration of H_2O_2, which, due to high toxicity, must be in strict accordance with the activity of antioxidant systems (Szanto et al., 2019). Normally, many biochemically unfavorable processes occur on the apical membrane of the thyrocyte, which makes it possible to limit the action of free radicals and avoid cell destruction (Carvalho and Dupuy, 2013). However, under pathological conditions, enzymatic systems are disrupted and their components become abnormally activated in the cytoplasm, and this, in turn, leads to functional and morphological disorders (Ohye and Sugawara, 2010). Inflammatory-dystrophic changes in tissues, in these conditions, are associated with the attack of free radicals from the internal environment of the body, where, for various reasons, their increased concentration arises and is maintained (Phaniendra et al., 2015). Free oxygen radicals adversely affect biological molecules such as lipids, proteins, and DNA (Lobo et al., 2010). However, one should not forget about the

important role of oxidative stress in the physiological adaptation and regulation of intracellular signaling (Burton and Jauniaux, 2011).

Oxidative stress may be a significant risk factor in the pathogenesis and progression of Hashimoto's thyroiditis and the development of complications (Mikulska et al., 2022). A deeper understanding of the nature of oxidative stress and its role in the development of autoimmune thyroid disorders may contribute to the identification of new methods for its assessment and the expansion of therapeutic ranges for this disease.

Paying attention to the relevance of a current issue, we decided to analyze changes in markers of oxidative stress (lipid peroxidation and oxidative modification of proteins) and the total antioxidant capacity in the blood of women and men with autoimmune Hashimoto's thyroiditis (HT).

Materials and methods

Participants. The participants of the study were recruited among patients of non-public Health Care Center U & O Zdrowie – Home-based long-term care (Lębork, Poland). A detailed medical history was taken, and a physical examination was performed on all participants. The Research Ethics Committee of the Regional Medical Commission in Gdańsk (Poland) approved the study (KB-32/18). All patients provided written informed consent before the start of the study procedures. This study was carried out in a group of 153 individuals. The group of women participating in the study consisted of 109 individuals (71.24 %), while the group of men consisted of 44 individuals (28.76 %). All persons were divided into two groups: 1) euthyroidism (n = 64; men – n = 18, and women – n = 46); and 2) Hashimoto's autoimmune thyroiditis with subclinical hypothyroidism (n = 89; men – n = 26, and women – n = 63).

The functioning of the thyroid gland was additionally verified by measuring the concentration of thyrotropin (TSH), free triiodothyronine (fT3), free tetraiodothyronine (thyroxine, fT4), and antibodies against thyroid peroxidase (anti-TPO). The concentration of thyrotropin, triiodothyronine, free thyroxine and the concentration of antibodies against thyroperoxidase in human serum were determined using the electrochemiluminescence method "ECLIA" on the immunological analyzer Elecsys Cobas e 411 (Hitachi, Japan). In each group of women and men with euthyroidism and Hashimoto's autoimmune thyroiditis with subclinical hypothyroidism, the 2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives of protein oxidative modification (OMP), and total antioxidant capacity (TAC) were determined.

Blood samples. For determination of thyrotropin, triiodothyronine, free thyroxine, and the concentration of antibodies against thyroperoxidase, the material for the study was blood collected from the cubital vein into special clot tubes (Serum ClotActivator). It was then centrifugated in a centrifuge for 3 minutes at 3,000 rpm. The resulting serum was transferred to properly labeled tubes and evaluated in a biochemistry analyzer for 18 minutes.

For the determination of oxidative stress biomarkers, venous blood samples (5 ml) were obtained from the capital vein of each participant using sterile disposable plastic syringes. Specimens were collected at the same standardized time to minimize any effect of diurnal variation. The blood samples were collected in tubes with K_3-EDTA anticoagulant (1.5 ± 0.25 mg/ml). The clear, non-hemolyzed supernatant plasma was separated using clean, dry disposable plastic syringes. Blood samples and plasma were stored at +4°C and used within 2 days for the analysis of biomarkers of oxidative stress.

The 2-Thiobarbituric acid reactive substances (TBARS) assay. The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the Kamyshnikov (2004) method for determining the malonic dialdehyde (MDA) concentration. This method is based on the reaction of the degradation of the lipid peroxidation product, MDA, with 2-thiobarbituric acid (TBA) under high temperature and acidity to generate a coloured adduct that is measured spectrophotometrically. The nmol of MDA per mL was calculated using $1.56 \cdot 10^5$ mM^{-1} cm^{-1} as the extinction coefficient.

The carbonyl derivatives of protein oxidative modification (OMP) assay. To evaluate the free radical-induced protein damage in blood samples, a content of carbonyl derivatives of protein oxidative modification (OMP) based on the spectrophotometric measurement of aldehydic and ketonic derivatives in the blood was performed. The rate of protein oxidative destruction was

estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine et al. (1990) and as modified by Dubinina et al. (1995). DNFH was used for determining the contents of carbonyl groups in soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

Measurement of total antioxidant capacity (TAC). The TAC level in samples was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm (Galaktionova et al., 1998). The sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The level of TAC in the sample (%) was calculated according to the absorbance of the blank samples.

Statistical analysis. The mean ± S.E.M. values were calculated for each group to determine the significance of the intergroup difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). The significance of differences between parameters (significance level, $p<0.05$) was examined using the Mann-Whitney U test and the Kruskal-Wallis test by rank (Zar, 1999). All statistical calculations were performed on separate data from each individual with STATISTICA 13.3 software (TIBCO Software, Polska).

Results and discussion

Malonic dialdehyde (MDA) is an end product of lipid peroxidation induced by ROS. This marker can be used to assess oxidative damage and measure whole-body or tissue oxidative stress (Torun et al., 2009; Erdamar et al., 2010). Elevated MDA levels are observed in tissues damaged by ROS as end products of peroxidation, which makes them markers of oxidative stress in the body (Ruggeri et al., 2021). The level of 2-thiobarbituric acid reactive substances (TBARS) in the blood of men and women with euthyroid state and autoimmune thyroiditis with subclinical hypothyroidism is presented in Fig. 1.

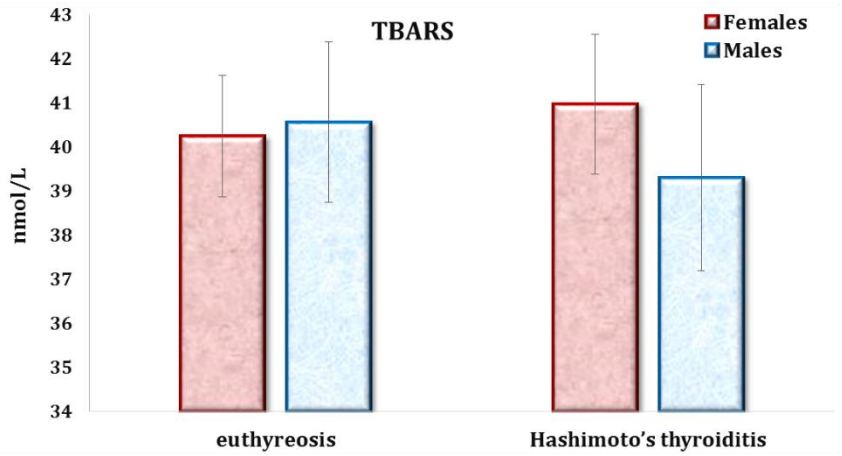

Fig. 1. The level of 2-thiobarbituric acid reactive substances (TBARS) in the blood of men and women with euthyroid state and autoimmune thyroiditis with subclinical hypothyroidism.

The results of our study showed a higher level of TBARS in the blood of women with HT (40.97 ± 1.58 nmol/L) compared to the values in euthyroid women (40.24 ± 1.38 nmol/L). In the group of men, other results were obtained, e.g. the level of TBARS in men with Hashimoto's disease was lower (39.30 ± 2.11 nmol/L) compared to the euthyroid group (40.56 ± 1.82 nmol/mL). The level of TBARS in the blood of women with HT was 1.8 % ($p > 0.05$) higher compared to women with euthyroidism. However, the level of TBARS in the blood of men with HT was 1.78 % ($p > 0.05$) lower than in the group of men with euthyroidism. Comparing the values of

TBARS in groups of euthyroid women and men, we can conclude that this level is at the same level in women (40.24 ± 1.32 nmol/L) and men (40.56 ± 1.82 nmol/L) (Fig. 1).

Carbonyl derivatives of proteins are stable products that are formed with the participation of amino acid residues of proline, arginine, lysine, and threonine with the formation of Michael adducts. Also, carbonyl derivatives of proteins can be formed with the participation of amino acid residues of lysine, cysteine, and histidine with products of lipid peroxidation. Moreover, the carbonylation of arginine and lysine is accompanied by the loss of one or more nitrogen atoms. In addition, they can be formed during glycation/glycoxidation of lysine amino groups. According to a number of researchers, carbonyl derivatives are formed during metal-catalyzed protein oxidation (Grimsrud, 2008). The most important consequence of protein oxidation is the inactivation of enzymes. Modification of proteins makes them more sensitive to proteolysis. An increase in the persistence of carbonyl proteins may be the result of a decrease in the activity of cellular protease systems (Dissmeyer et al., 2018).

The level of aldehydic and ketonic derivatives of oxidative modification of proteins in the blood of men and women with euthyroid state and autoimmune thyroiditis with subclinical hypothyroidism is presented in Figures 2 and 3.

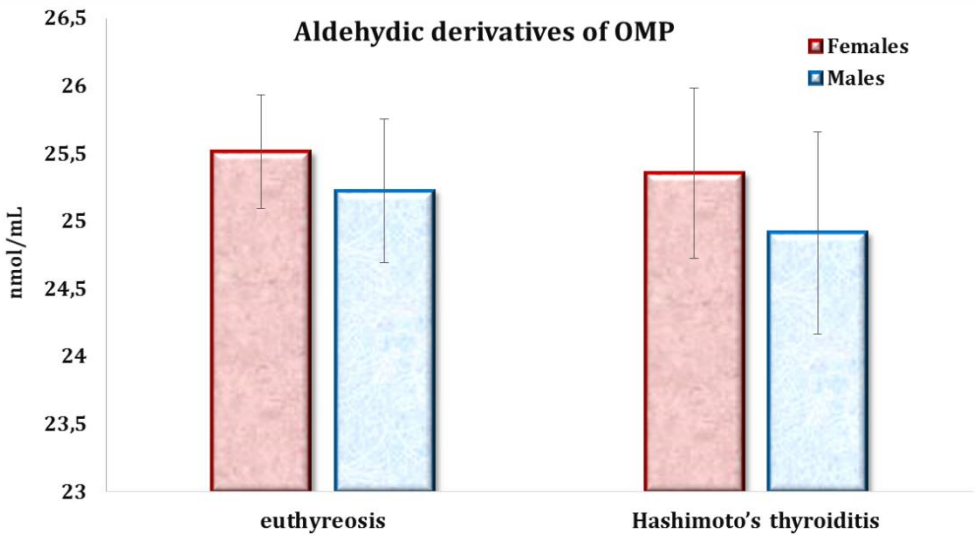

Fig. 2. The level of aldehydic derivatives of oxidative modification of proteins in the blood of men and women with euthyroid state and autoimmune thyroiditis with subclinical hypothyroidism.

As a result of the current research, a slightly lower level of aldehydic derivatives of oxidative modification of proteins was obtained in the blood of women with autoimmune thyroiditis (25.35 ± 0.63 nmol/mL) compared to the values in euthyroid women (25.51 ± 0.42 nmol/mL). mL). In the group of men, similar results were obtained, i.e. the level of aldehydic derivatives of OMP in men with HT was (24.91 ± 0.75 nmol/mL), compared to the group of men with euthyroidism (25.22 ± 0.53 nmol/mL). The level of aldehydic derivatives of OMP in women and men with HT was respectively lower by 0.7 % ($p > 0.05$) and 1.2 % ($p > 0.05$) compared to the group of women and men with euthyroidism. Comparing the values of aldehydic derivatives of oxidative modification of proteins in euthyroid women and men, we can conclude that the level of aldehydic derivatives of OMP is at the same level in women (25.51 ± 0.42 nmol/mL) and men (25.22 ± 0.53 nmol/mL) (Fig. 2).

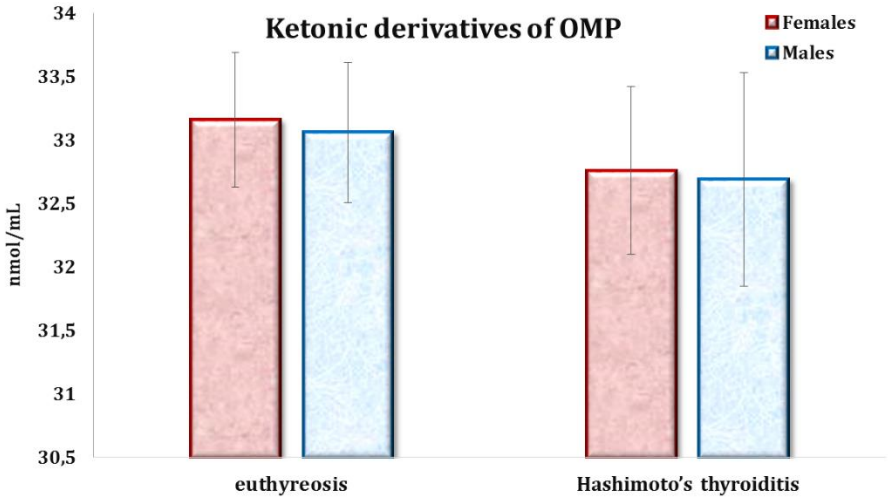

Fig. 3. The level of ketonic derivatives of oxidative modification of proteins in the blood of men and women with euthyroid state and autoimmune thyroiditis with subclinical hypothyroidism.

The results of our study showed a lower level of ketonic derivatives of OMP in the blood of women with HT (32.76 ± 0.66 nmol/mL) compared to the values in euthyroid women (33.16 ± 0.53 nmol/mL). Similar results were obtained in the group of men. In men with Hashimoto's disease, the level of ketonic derivatives of oxidative protein modification was lower (32.69 ± 0.84 nmol/mL) compared to the group of euthyroid men (33.06 ± 0.66 nmol/mL). The level of ketonic derivatives of OMP in women and men with HT was lower by 1.2% ($p > 0.05$) and 1.1 % ($p > 0.05$), respectively, compared to the euthyroid group of women and men. Comparing the values of ketonic derivatives of OMP in euthyroid men and women, we can conclude that the level of ketonic derivatives of oxidative modification of proteins is at the same level in women (33.16 ± 0.53 nmol/mL) and men (33.06 ± 0.66 nmol/mL) (Fig. 3).

Total antioxidant capacity (TAC) is a parameter that indicates the overall ability of the body to neutralize oxidants. It takes into account all antioxidants contained in body fluids, including exogenous and endogenous compounds (Marrocco et al., 2017). In turn, the total oxidation state (TOS) is based on the oxidation of ferrous ions to ferric ions in the presence of various oxidants. It reflects the degree of oxidation of body fluids, represented by the level of radicals (Rovcanin et al., 2016). The oxidative stress index (OSI) is a measure calculated as the ratio of the total oxidation state to the total antioxidant response (TAR) and therefore represents the overall degree of oxidation of the body (Ates et al., 2018).

The level of total antioxidant capacity in the plasma of men and women with euthyroid state and autoimmune thyroiditis with subclinical hypothyroidism is presented in Fig. 4.

As a result of the current studies, a slightly higher level of total antioxidant capacity in the plasma of women with autoimmune thyroiditis was obtained (45.27 ± 1.98 %) compared to the value in euthyroid women (42.48 ± 2.11 %). In the group of men, similar results were obtained, i.e. the level of TAC in men with HT was (48.56 ± 3.38 %), compared to the group of men with euthyroidism (47.54 ± 2.12 %). TAC levels in the plasma of men and women with HT were higher by 6.5 % ($p > 0.05$) and 2.1 % ($p > 0.05$), respectively, compared to euthyroid women and men. Comparing the values of total antioxidant capacity in the euthyroid groups of women and men, we can conclude that the plasma TAC level was at a lower level in women (42.48 ± 2.11 %) compared to men (47.54 ± 2.12 %) (Fig. 4).

The level of total antioxidant capacity in the erythrocytes of men and women with euthyroid state and autoimmune thyroiditis with subclinical hypothyroidism is presented in Fig. 5.

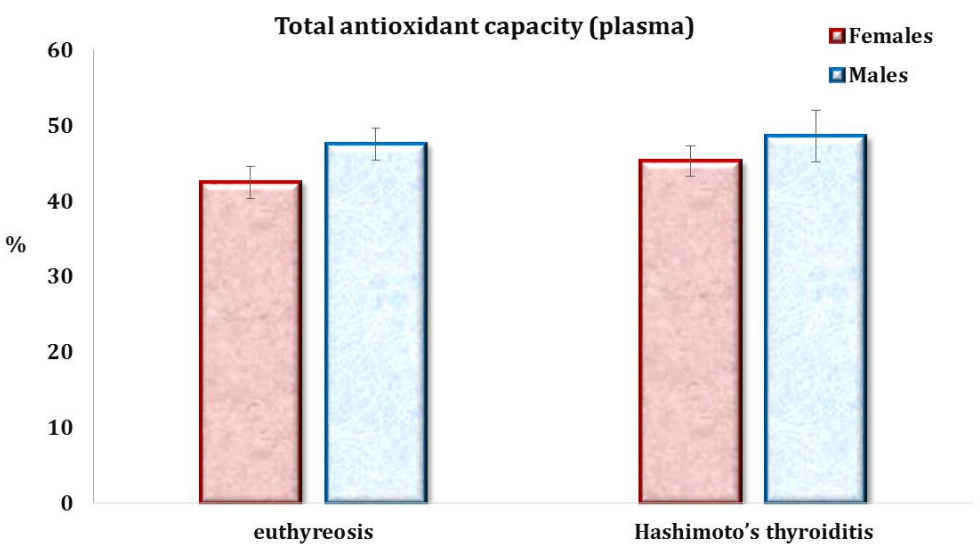

Fig. 4. The level of total antioxidant capacity in the plasma of men and women with euthyroid state and autoimmune thyroiditis with subclinical hypothyroidism.

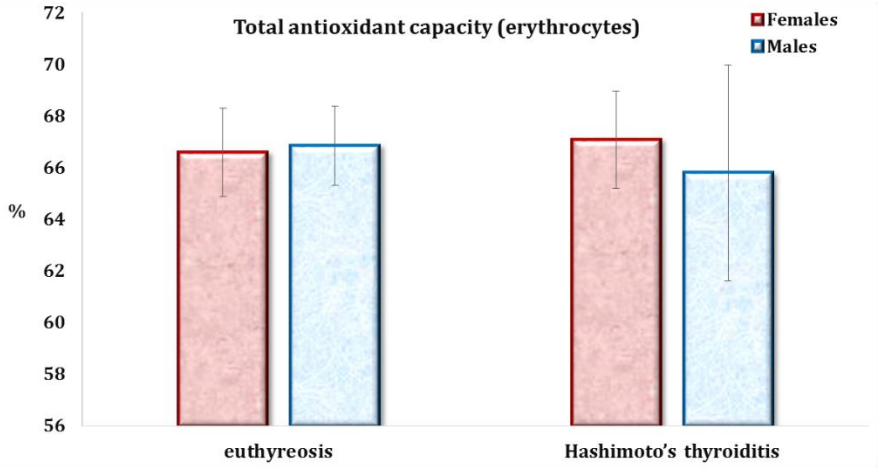

Fig. 5. The level of total antioxidant capacity in the erythrocytes of men and women with euthyroid state and autoimmune thyroiditis with subclinical hypothyroidism.

As a result of the current research, a slightly higher level of total antioxidant capacity was obtained in the erythrocytes of women with autoimmune thyroiditis (67.08 ± 1.89 %) compared to the values in women with euthyroidism (66.59 ± 1.71 %). However, in the group of men, the level of TAC was slightly lower (65.80 ± 4.18 %) compared to the group of men with euthyroidism (66.85 ± 1.54 %). The level of TAC in the erythrocytes of women with HT was higher by 0.8 % ($p > 0.05$) and lower by 1.6 % ($p > 0.05$) in men compared to the group of women and men with euthyroidism, respectively. Comparing the TAC values in the erythrocytes of women and men with euthyroidism, we can conclude that the level of TAC in erythrocytes was at the same level in women (66.59 ± 1.71 %) and men (66.85 ± 1.54 %) (Fig. 5).

In the current study, we analyzed changes in levels of oxidative stress markers (lipid peroxidation and oxidative modification of proteins) and the total antioxidant capacity in

the blood of women and men with Hashimoto's thyroiditis. As a result of the current research, statistically significant changes in levels of oxidative stress markers were not observed. In women with HT, elevated TBARS levels with simultaneously increased TAC levels in the plasma and erythrocytes were observed. Additionally, levels of aldehydic and ketonic derivatives of oxidative modification of proteins in the blood of women with HT were lower compared to the women with the euthyroid state. In men with HT, levels of markers of oxidative stress (except TAC levels in the plasma) were lower compared to those obtained in men with the euthyroid state (Fig. 1-5).

The results of studies on the relationship between hypothyroidism and oxidative stress in humans are conflicting (Rybakova et al., 2019). It has been suggested that in patients with AIT and hypothyroidism, the prooxidant environment may play a role in the development of atherosclerosis (Papadopoulou et al., 2020). The increase in oxidative stress can be explained not only by a decrease in the concentration of antioxidants but also by a change in lipid metabolism since a significant relationship was found between the concentration of malonic dialdehyde (MDA) and LDL, the content of total cholesterol, and triglycerides. Increased oxidative stress in both hypothyroid and subclinical hypothyroidism states can be explained by both the insufficient increase in the antioxidant status and the altered lipid metabolism in these cases (Torun et al., 2009). Other studies have also confirmed an increase in MDA in both overt hypothyroidism and subclinical hypothyroidism (Torun et al., 2009; Haribabu et al., 2013). Morawska et al. (2020) observed that total antioxidant potential (TAC) was significantly lower (by 82 %), while total oxidant status (TOS), oxidative stress index (OSI), and the level of oxidation products of proteins (AGEs) and lipids (lipid hydroperoxides) were significantly higher in HT patients compared to the control group. Moreover, the saliva of euthyroid patients with HT also demonstrated a reduced antioxidant potential (Morawska et al., 2020).

It remains a debatable question whether oxidative stress is associated with hypothyroidism as such or whether it is associated with a change in the lipid spectrum due to thyroid dysfunction. For example, Santi et al. (2012) described oxidative stress in subclinical hypothyroidism (indicated by decreased arylesterase and elevated TBARS and catalase activity) but associated it with the influence of hypercholesterolemia in thyroid dysfunction. Secondary hypercholesterolemia to thyroid dysfunction and not hypothyroidism per se appears to be associated with oxidative stress in subclinical hypothyroidism (Santi et al., 2012).

Another study (Öztürk et al., 2012) conducted on patients with subclinical hypothyroidism in the outcome of AIT did not reveal differences in the concentration of MDA in patients with hypothyroidism and in the control group; however, in patients with hypothyroidism, the activity of the pro-oxidant 2,2'-azobis-(2-amidinopropane) hydrochloride, which stimulates MDA generation, was increased. According to the results of the study, the increase in MDA did not differ in patients with subclinical hypothyroidism compared with the control group, while it differed significantly from the control in patients with overt hypothyroidism (Öztürk et al., 2012). However, the results of the study should be interpreted with caution, since both local and systemic inflammation are present in autoimmune thyroiditis (Rybakova et al., 2019).

Recent studies have shown that high oxidative stress is associated with the severity and progression of Hashimoto's thyroiditis from euthyroidism to overt hypothyroidism (Athes et al., 2015, 2018). Athes et al. (2015) conducted a study with newly diagnosed hypertension (31 in each stage: euthyroid, subclinical, and overt hypothyroidism) without treatment and 31 healthy volunteers. The level of oxidative stress was higher at all stages of Hashimoto's thyroiditis compared with the control group, and a negative correlation was observed between the total antioxidant balance and thyroid autoantibodies. Moreover, total oxidant status and oxidative stress index levels were elevated and total antioxidant status (TAS) was reduced in patients with overt hypothyroidism compared with other groups. These results suggest that oxidative stress continues to increase during an exacerbation of hypothyroidism in hypertensive patients (Athes et al., 2015). A later study, also by Ates et al. (2018) showed that after 9 months of follow-up of patients with euthyroid and subclinical hypothyroidism without treatment, 17.5 % of them developed overt hypothyroidism. total oxidant status and oxidative stress index were

higher in patients who developed overt hypothyroidism than in those who did not. In addition, a positive correlation was observed between oxidant parameters and thyroid autoantibodies. The authors concluded that oxidative stress may be a risk factor for the development of overt hypothyroidism in hypertensive patients (Athes et al., 2018).

Nekrasova et al. (2011) investigated lipid and protein peroxidation in autoimmune thyroiditis (AT) and subclinical hypothyroidism (SH), as well as evaluated its correlation for arterial stiffness and relationship to replacement treatment. The study included 85 women; 32 of them had no thyroid pathology (group 1, controls) and 53 had AT and SH (group 2). Free-radical protein and lipid oxidation (FRO), serum antioxidant activity (AOA), and arterial elasticity parameters were investigated. In addition, it compared different categories of the patients included in group 2: patients having euthyroidism (n = 18) and subclinical hypothyroidism (n = 35); AT patients with TSH less (n = 26) and not less (n = 27) than the median of group values (~~6 mU/ml). AT patients demonstrated a higher oxidative protein modification rate (OMB) which was confirmed by a 75% elevation of OMB 363 nm index (p = 0,049) and greater activity of lipid peroxidation (LPO) confirmed by an 11 % elevation of luminescence intensity index Imax (p = 0,035) but no increase in AOA, catalase and superoxide dismutase activity (p > 0,05). Oxidative stress severity was positively associated with TSH; dyslipidaemia had a stronger influence on oxidative stress. The proportions of women with the relative disparity between elevated malondialdehyde (MDA) and depressed antioxidant activity (AOA), were significantly higher in AT and SH groups (p < 0,05). The most significant difference between oxidative stress parameters was found among patients having TSH less and not less than 6 mU/ml. The subgroup with higher TSH was found to have higher indexes of endogenous intoxication and LPO products values (the increase of EI254nm, EI274nm, EI294nm, triene conjugates by 18 %; 19 %; 29 % and 38 % respectively, p < 0,05) (Nekrasova et al., 2011).

Among all the hormones that act on the antioxidant system, thyroid hormones play a particularly important role, since both hyperthyroidism and hypothyroidism are associated with oxidative stress (Chakrabarti et al., 2016; Mancini et al., 2016). However, the oxidative stress mechanisms in these two states are different: increased reactive oxygen species (ROS) production in hyperthyroidism and low availability of the antioxidant system in hypothyroidism (Kochman et al., 2021). Thyroid hormones as such can act as oxidants and damage DNA, probably through a phenolic group that is similar in activity to steroidal estrogens (Villanueva et al., 2013; Mancini et al., 2016). Other mechanisms may be involved, in particular, increased expression of nitric oxide synthase (NOS) with excessive production of NO and activation of hepatic transcription factor NF-kB, followed by an increase in the concentration of cytokines that induce ROS products (Tapia et al., 2003; Barreiro Arcos et al., 2006; Mancini et al., 2016; Rybakova et al., 2019).

On the other hand, the mechanisms regulated by thyroid hormones carry out a fine regulation of the oxidative status through feedback. Among them, the role of proteins UCP-2 and UCP-3 (uncoupling proteins 2 and 3) is emphasized. These molecules have antioxidant activity (Lanni et al., 2003; Zaninovich, 2005). However, only triiodothyronine (T3) seems to regulate UCP, while thyroxine (T4) has no effect (Lanni et al., 2003). Studies have shown that thyroid hormones affect the lipid metabolism of rat tissues and, consequently, their susceptibility to oxidative stress. However, conflicting effects have been noted from exposure to T3 and T4. In rat liver, T3-induced hyperthyroidism has been found to be associated with altered lipid peroxidation indices, including elevated levels of 2-thiobarbituric active substances (TBARS) and lipid hydroperoxides, which are by-products of lipid peroxidation (Venditti et al., 1997, 1999). In contrast, no change in the increase in TBARS was found in the homogenized liver of hyperthyroid rats treated with T4 over a 4-week period (Huh et al., 1998). Significant increases in the amount of TBARS or lipid hydroperoxides were observed in the testicles of hyperthyroid adult rats (Zamoner et al., 2007). The T3 treatment produced an imbalance in their testicular redox status, reflected by a significant increase in the amount of TBARS and protein carbonyl content in the testicular homogenates of 20-day-old rats (Kamel and Hamouli-Said, 2018).

Cheserek et al. (2015) determined whether there was increased oxidation of lipids and proteins in SCH, and examined their association

with lipids and thyroid hormones. The results of these researchers revealed that oxidative stress was increased in subclinical hypothyroidism as evidenced by the elevated lipid peroxidation product, malondialdehyde, while protein oxidation was absent. Thus, the reduction of oxidative stress may be beneficial in patients with subclinical hypothyroidism.

Conclusions

In the current study, we analyzed changes in levels of oxidative stress markers (lipid peroxidation and oxidative modification of proteins) and the total antioxidant capacity in the blood of women and men with Hashimoto's thyroiditis. As a result of the current research, statistically significant changes in levels of oxidative stress markers were not observed. In women with HT, elevated TBARS levels with simultaneously increased TAC levels in the plasma and erythrocytes were observed. Additionally, levels of aldehydic and ketonic derivatives of oxidative modification of proteins in the blood of women with HT were lower compared to the women with the euthyroid state. In men with HT, levels of markers of oxidative stress (except TAC levels in the plasma) were lower compared to those obtained in men with the euthyroid state.

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