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THE STATE OF ANTIOXIDANT DEFENSE SYSTEM IN YOUNG PERSONS WITH GASTROESOPHAGEAL REFLUX DISEASE AND AUTOIMMUNE THYROIDITIS

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Key words: *gastroesophageal reflux disease, autoimmune thyroiditis, total antioxidant activity, manganese superoxide dismutase, Klotho protein*
Ключові слова: *гастроезофагеальна рефлюксна хвороба, автоімунний тиреоїдит, загальна антиоксидантна активність, марганець супероксиддисмутаза, протеїн Клото*
Ключевые слова: *гастроэзофагеальная рефлюксная болезнь, аутоиммунный тиреоидит, общая антиоксидантная активность, марганец супероксиддисмутаза, протеин Клото*

Abstract. The state of antioxidant defense system in young persons with gastroesophageal reflux disease and autoimmune thyroiditis. Kovalyova O.M., Chukhrienko N.D., Pasiieshvili T.M., Pasiyeshvili L.M., Zhelezniakova N.M. *Aim of research was assessment of the levels of antioxidant biomarkers associated with mitochondrial function in young patients with gastroesophageal reflux disease (GERD) and autoimmune thyroiditis*

(AIT). This study included 165 patients of them 120 patients with GERD and AIT - the main group, 45 patients with isolated GERD - the comparison group. The examined contingent was presented by students aged 18 to 25 years. The control group consisted of 20 healthy individuals of corresponding gender, age and social status (students). Total antioxidant activity (TAS-TAC) was determined in blood serum of study persons with enzyme immunoassays (ELISA, Elabscience, USA), levels of manganese superoxide dismutase (MnSOD) – with enzyme immunoassays (ELISA, Elabscience, USA) and Klotho protein - with enzyme immunoassays (ELISA, Elabscience, USA). Statistical data processing by the Statistica Basic Academic 13 for Windows En local was made. In examined patients with esophageal and thyroid pathologies the decline of total antioxidant activity has been revealed. The MnSOD level in patients with comorbidity of GERD and AIT and isolated GERD was significantly higher as compare to control group. Significant increasing of Klotho protein in serum of young patients was observed. We assessed imbalance between decline of extracellular antioxidants and activation of mitochondrial antioxidants which is more pronounced in combination of diseases. The increase of biomarkers of mitohondrial antioxidant defense system with non-specific citoprotection mechanism in patients with GERD provides the basis to consider MnSOD and Klotho protein as prognostic indicator of clinical outcome of disease in young age. Under combination of GERD and AIT the tendency to overexpression of MnSOD and depression of total antioxidant activity has been revealed, this may cause the deterioration of mitochondrial function.

Реферат. Стан антиоксидантної системи в молодих пацієнтів з гастроезофагеальною рефлюксною хворобою та автоімунним тиреоїдитом. Ковальова О.М., Чухрієнко Н.Д., Пасієшвілі Т.М., Пасієшвілі Л.М., Железнякова Н.М. *Метою дослідження було визначення в пацієнтів молодого віку з гастроезофагеальною рефлюксною хворобою (ГЕРХ) та автоімунним тиреоїдитом (АІТ) рівня антиоксидантних біомаркерів, що асоціюються з функцією мітохондрій. Дослідження включало 165 хворих, серед яких 120 хворих з ГЕРХ і АІТ-основна група, 45 хворих з ізольованою ГЕРХ – група порівняння. Досліджуваний контигент представлено студентами у віці від 18 до 25 років. Контрольну групу склали 20 здорових осіб, що відповідали за статтю, віком та соціальним статусом (студенти). Загальна антиоксидантна активність (TAS-TAC) у сироватці крові досліджуваних визначалась імуноферментним методом з використанням тест-системи (ELISA, Elabscience, США), рівень марганця супероксиддисмутази (MnSOD) – імуноферментним методом (ELISE, Elabscience, США), рівень протеїна Клото (Klotho protein) - імуноферментним методом (ELISA, Elabscience, США). Статистичний аналіз результатів дослідження було проведено за допомогою системи Statistica Basic Academic 13 for Windows En локальна. У досліджених хворих з патологією стравоходу та щитоподідної залози визначено зниження загальної антиоксидантної активності порівняно зі здоровими особами. Рівень марганцю супероксиддисмутази у хворих з коморбідним перебігом ГЕРХ та АІТ та хворих з ізольованою ГЕРХ достовірно перевищував показники контрольної групи. Відзначається достовірне збільшення рівня протеїну Клото в сироватці крові хворих молодого віку. Встановлено дисбаланс між ступенем зниження позаклітинних антиоксидантів та активацією мітохондріального антиоксиданта, що найбільш виражено у хворих при сполученні захворювань. Підвищення у хворих маркерів мітохондріального антиоксидантного захисту з неспецифічним механізмом цитопротекції дає підставу для використання марганцю супероксиддисмутази та протеїну Клото як прогностичних індикаторів клінічного перебігу захворювання в молодому віці. При коморбідності ГЕРХ та АІТ встановлена тенденція до значного підвищення продукції марганцю супероксиддисмутази на фоні зниження загального антиоксидантного захисту, що може призводити до погіршення мітохондріальної функції.*

Modern clinic of internal medicine is characterized by comorbidity which means the combination of some diseases in one patient. Among these diseases gastroesophageal reflux disease (GERD) and autoimmune thyroiditis (AIT) are ranked as the most common diagnoses in outpatient care.

The spreading of GERD is tremendously increasing worldwide resulting in adverse medical and economic consequences, its impact on healthcare cost is reported to be extremely high [5]. The symptoms of GERD may appear in young age as dangerous prognostic feature of more serious condition in future known as Barrett's esophagus. Another disease that often occurs at a young age is AIT. Both diseases may result from a multifactorial mechanisms with general pathological process related to the classical laws of inflammation.

Systemic inflammatory response syndrome of non-infection origin has recently come to be recognized as a fundamental clinical problem due to wide involving in pathogenesis of numerous diseases. Under condition of inflammation overproduction of reactive oxygen species (ROS) appear, thus a lot of diseases of internal organs are accompanied by formation of oxidative stress with damage to the biological macromolecules and cell membranes [11]. Synthesis of thyroid hormones and regulation of their metabolism involve free radicals that may affect redox balance in the body. Thyroid disorders causing variations in the levels of thyroid hormones may alter cellular oxidative stress [2].

The human body has several mechanisms to counteract oxidative stress by producing endogenous antioxidants which act as "free radical scavengers"

by preventing and repairing damages caused by ROS [10]. The important center for the metabolic activities of the body that involves ROS production is mitochondria [3]. Due to the chronic excessive ROS in mitochondria resulted from inflammation, the affection of mitochondrial DNA starts as well as direct damage to cell structure and function [7].

Mitochondria are not only the main sites of ROS production but also important organelles in the antioxidant system. The major ROS detoxifying enzyme of cells localized in mitochondria is superoxide dismutase (SOD) which is a metalloenzyme and hence, requires a metal cofactor for its activity named manganese superoxide dismutase (MnSOD) [14]. An important role in maintaining antioxidant defence during H_2O_2-induced oxidative stress plays newly discovered Klotho protein [15].

Nevertheless in previous studies there are insufficient data relevant to mitochondrial antioxidant defense system in patients with gastrointestinal disorders and thyroid disease. In order to clarify this problem we selected MnSOD and Klotho protein in blood serum of young persons as indicators of mitochondrial antioxidant system.

The purpose: assessment of the state of antioxidant defense system in young patients with GERD and AIT based on activity of biomarkers associated with mitochondrial function.

MATERIALS AND METHODS OF RESEARCH

This study included 165 patients, of them 120 patients with GERD and AIT – the main group, 45 patients with isolated GERD – the comparison group. The examined contingent was presented by university students aged 18 to 25 years. Median age in main group was 21.9±2.7 years and 21.2±2.4 years in comparison group; 93 patients (77.5%) in group with GERD and AIT and 34 patients (75.6%) with isolated GERD were women, 27 (22.5%) and 11 (24.4%) were men. The anamnesis of GERD and AIT did not exceed 3 years. The control group consisted of 20 healthy individuals, corresponding to gender, age and social status (students).

This research was conducted in compliance with all relevant diagnostic and treatment standards of the requirements for the ethical component of clinical trials (GCP, 1997). Before the study, patients were informed about the essence of the study, its purpose and possible results. All study participants provided written informed consent. This study was approved by the local ethics committee according to the recommendations of the ethical committees for biomedical research, Ukrainian legislation on health protection, the 2000 Helsinki Declaration and the directives of the European Partnership 86/609 on the participation of people in biomedical research.

Verification of the GERD diagnosis was performed according to the recommendation of the Montreal Consensus (2006), “Protocols for the management of patients ...” with this nosology and ICD-10”. The morphological form of the disease was revaeled during esophagogastroduodenoscopy («Fuginon» system, Japan) according to the recommendations of the Los Angeles classification.

Verification of AIT was performed on the basis of data from an ultrasound examination of the thyroid gland (Mindray DC-60 Exp, China) and evaluation of test results for antibodies to thyroid peroxidase and thyroglobulin; thyroid function was assessed by the levels of thyroid stimulating hormone, thyroxine and triidothyronine. Euthyroid state was in all cases of autoimmune thyroiditis.

Total Antioxidant Activity (Total Antioxidant Status and Total Antioxidant Capacity – TAS-TAC) was determined in blood serum of study persons with enzyme immunoassays (ELISA, Elabscience, USA) analyzer Labline-80. Levels of MnSOD in blood serum were determined with enzyme immunoassay analyzer Labline-90 (ELISA, Elabscience USA) and Klotho protein was determined in blood serum with enzyme immunoassay using a commercial test system manufactured by Elabscience (ELISA, USA) according to the instructions attached to the kit.

Statistical data processing was made by the Statistica Basic Academic 13 for Windows En local general-purpose software package (License Number: 139-956-866). The significance of differences was calculated using the Student's test (t). The relationship between the studied parameters was assessed using the Pearson correlation analysis (r). The critical level of statistical significance in checking all hypotheses was taken at <5% ($p<0.05$) [1].

RESULTS AND DISCUSSION

Total antioxidant activity in blood serum of study patients and control persons is presented in Table 1.

The present study demonstrates that patients with GERD and AIT and patients with isolated GERD have decreased levels of total antioxidant activity as compared to persons of control group. The total antioxidant activity in GERD and AIT patients was lower than in patients with isolated GERD, the difference is statistically significant between groups.

The levels of MnSOD in blood serum of study patients and control persons are presented in Figure 1.

Patients had significantly higher serum levels of MnSOD compared to control persons. In patients with combined course of GERD and AIT mediana and interpercentil deviation of MnSOD was 9.1965 (7.2480; 11.6385) ng/ml which is two times higher than the control indices – 4.4720 (3.7010;

5.2325) ng/ml (U=386, p<0.01), and significantly exceeded the indices of the comparison group (7.1700 (6.1056; 8.1948) ng/ml (U=108, p<0.01). The indices of the group with isolated GERD also exceeded the indices of the control group. (U=276, p<0.01).

Table 1

Total antioxidant activity (TAS-TAC) of study patients and control persons

Patients/ indicators	TAS-TAC, µmol/l	Significance of differences compared with control group*	Significance of differences between groups*
GERD and AIT	305.2570 (160.1890; 425.1080)	U=376 p<0.01	U=1557 p<0.01
GERD	439.4680 (311.5332; 585.6220)	U=284.5 p<0.01	
Control group	517.8108 (419.1486; 809.2243)		

Note. *p<0.05 – the difference is statistically significant between groups.

The levels of Klotho protein in blood serum of study patients and control persons are presented in Figure 2.

Klotho protein levels was significantly higher in both study groups of patients compared to healthy individuals (0.4958 (0.3679; 0.6098) ng/ml). In group with isolated GERD it was 0.6130 (0.4612; 0.7630) ng/ml (U=279.5, p<0.01). Levels of Klotho protein in group with comorbidity GERD and AIT were 0.8070 (0.6110; 1.1840) ng/ml and exceeded levels of the control group (U=320.5, p<0.01) and of the comparison group (U=1570, p<0.01). We found direct correlation between the levels Klotho protein and MnSOD in the main group (r=0.724).

According to presented data in young patients with GERD and AIT total antioxidant activity decreasies which is primarily extracellular chain antioxidant representing the functional sum of antioxidants in plasma [8].

Total antioxidant activity is one of the feature of oxidative stress defined as an imbalance between radicals and antioxidant defense and is involved into pathophysiological mechanism of different diseases. Previously studies suggested that inflammatory cytokines and oxidative stress induced by the mixed reflux of gastric acid and duodenal juice play an important role in the development of esophagitis [13].

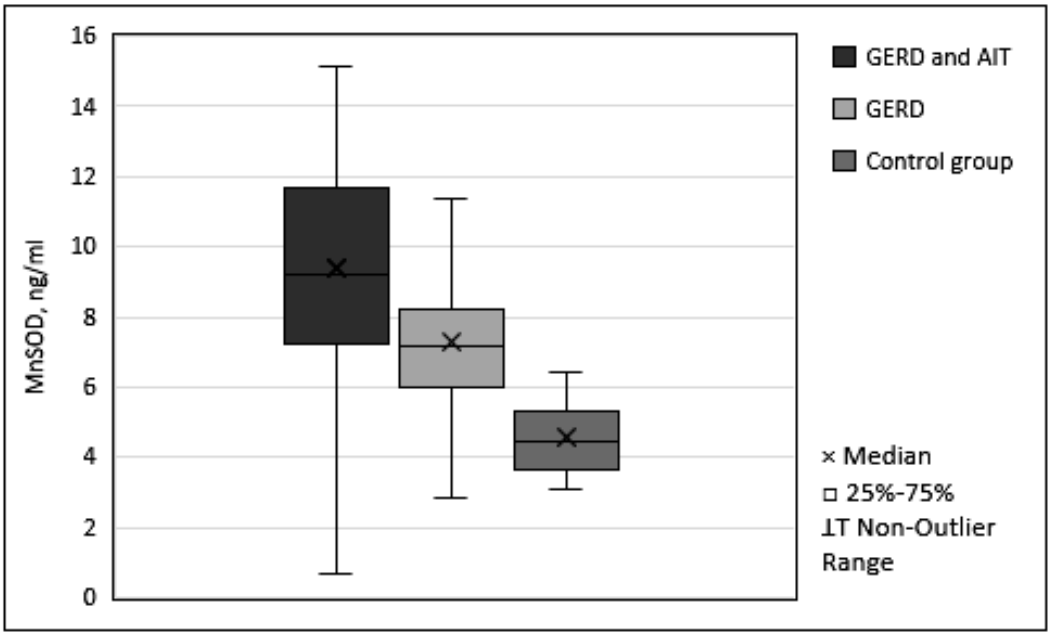

Fig. 1. Levels of MnSOD in patients of study and control group

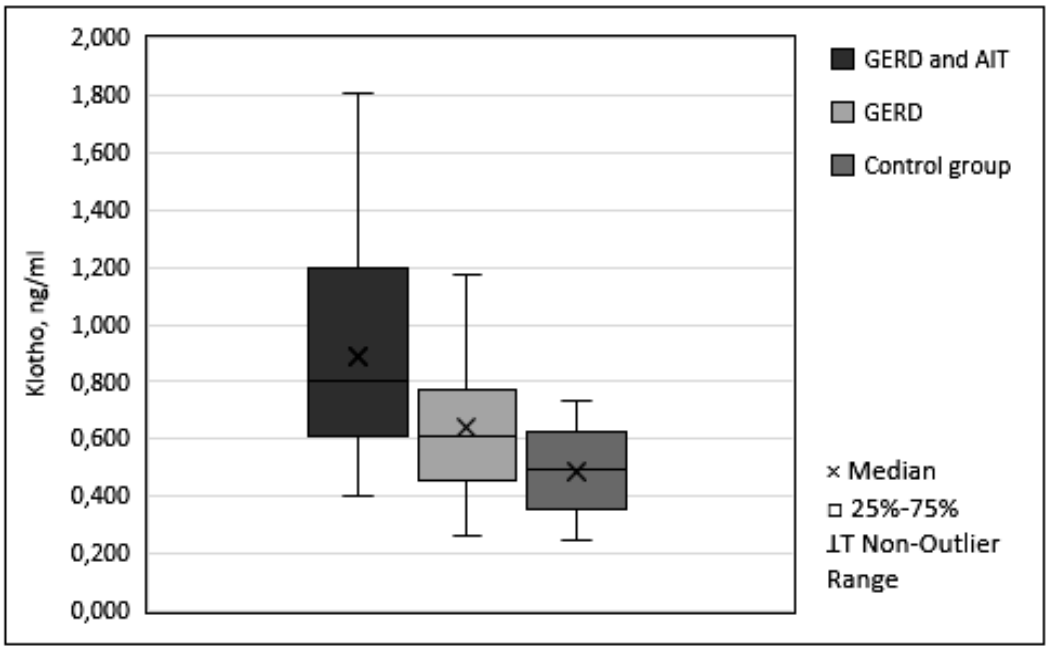

Fig. 2. Levels of Klotho protein in individuals of study and control groups

Oxidative stress can be related to hormonal disturbances due to the modification of synthesis, activity and metabolism of thyroid hormones. Thyroid disorders cause influence of the levels of antioxidant status, decline capability to prevent oxidative stress [12].

Thus, in our study we showed that the comorbidity of GERD and AIT in patients causes the failure of the extracellular phase of the antioxidant defense and various decline of control over the toxic radicals. It is known that stressors not only damage epithelial cells, but also causes free radical generation in their mitochondria causing dramatic effects on cellular function, organ injury and disturbance. [4].

High serum levels of MnSOD in patients of both studied group significantly exceeded the levels in control group patients. These data relevant to activation of intracellular antioxidant system seem to be an adaptive response to suppression of extracellular antioxidant system in patients with esophagitis and thyroiditis. MnSOD regulates mitochondrial function by decreasing ROS production, protect from pro-oxidant and pro-apoptotic stimuli as well as from ischemic damage [6, 9].

Recently it has been revealed that increased activities of antioxidant enzymes (superoxide dismutase, catalase and heme oxygenase-1) may be due to Klotho protein which induces the expression of the manganese superoxide dismutase and acts as ROS scavenge, poses antioxidant and anti-apoptotic effects in response to oxidative damage to cells, inhibites TNF-α, IL-6 [15, 16].

As a result of our study we have received the parallel increasing the levels of mitochondrial SOD and Klotho protein in blood serum in the group of patients with comorbidity and isolated pathology as compared to control group. Correlation between MnSOD and Klotho protein confirmed the participation of Klotho protein in antioxidant system due to stimulation of which resistance to oxidative stress increases and cytoprotection is provided.

Based on experimental and clinical studies it is known that expression of MnSOD provides positive effect and protects of a variety of cell lines from oxidative stress in case of adequate activity of antioxidant enzyme like superoxide dismutase, catalase and glutathione peroxidase which can prevent oxidation by reducing the rate of chain initiation. On the other hand in certain circumstances in reduced extracellular antioxidant activity MnSOD may lose their antioxidative ability and even act as a pro-oxidant which can generate toxic reactive oxygen forms, may work as a peroxidase, using H_2O_2- to promote mitochondrial damage.

CONCLUSIONS

1. An isolated gastroesophageal reflux disease and its combination with the autoimmune thyroiditis is accompanied by a decrease in total antioxidant activity and an increase in MnSOD levels.
2. Gastroesophageal reflux disease and autoimmune thyroiditis in young patients is accompanied with elevation of Klotho protein in blood serum which is responsible for activation of the antioxidant

enzyme of manganese superoxide dismutase and hence increases resistance to oxidative stress and provides cytoprotection.

3. The imbalance between extracellular and intracellular antioxidant defense system is more pronounced in patients with gastroesophageal reflux disease and autoimmune thyroiditis.

4. Thyroid disorders that accompany gastroesophageal reflux disease are characterized by the tendency to MnSOD overexpression, which may have negative effects.

Conflict of interests. The authors declare no conflict of interest.

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