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V.M. Zhdan, T.M. Pasiieshvili[1], N.M. Zhelezniakova[1], O.M. Kovalyova[1], L.M. Pasiieshvili[1]
Ukrainian Medical Stomatological Academy, Poltava
[1]Kharkiv National Medical University, Kharkiv

KLOTHO PROTEIN AND MITOCHONDRIAL SUPEROXIDE DISMUTASE IN YOUNG PERSONS WITH GASTROESOPHAGEAL REFLUX DISEASE AND AUTOIMMUNE THYROIDITIS

e-mail: pasotoma2017@gmail.com

The purpose of the work was to study the levels of the Klotho protein and mitochondrial superoxide dismutase in young patients with a comorbid course of gastroesophageal reflux disease and autoimmune thyroiditis. Study conducted 165 students: 120 with gastroesophageal reflux disease and autoimmune thyroiditis and 45 with isolated gastroesophageal reflux disease. Klotho protein and mitochondrial superoxide dismutase were studied in blood serum by enzyme immunoassay. The study of mitochondrial superoxide dismutase and Klotho protein showed a significant increase in these indicators in patients with gastroesophageal reflux disease and autoimmune thyroiditis in comparison with the control group and the group with isolated gastroesophageal reflux disease. The Klotho protein and mitochondrial superoxide dismutase can be used as biomarkers of gastroesophageal reflux disease progression in young patients with concomitant autoimmune thyroiditis.

Key words: gastroesophageal reflux disease, autoimmune thyroiditis, Klotho protein, mitochondrial superoxide dismutase.

В.М. Ждан, Т.М. Пасієшвілі, Н.М. Железнякова, О.М. Ковальова, Л.М. Пасієшвілі

БІЛОК КЛОТО І МІТОХОНДРІАЛЬНА СУПЕРОКСИДДИСМУТАЗА У ОСІБ МОЛОДОГО ВІКУ З ГАСТРОЕЗОФАГЕАЛЬНОЮ РЕФЛЮКСНОЮ ХВОРОБОЮ ТА АВТОІМУННИМ ТИРЕОЇДИТОМ

Метою роботи було вивчення рівня білка Клото і мітохондріальної супероксиддисмутази у молодих пацієнтів з коморбідним перебігом гастроезофагеальної рефлюксної хвороби і автоімунного тиреоїдиту. У дослідженні взяли участь 165 студентів: 120 з гастроезофагеальною рефлюксною хворобою і автоімунний тиреоїдитом і 45 з ізольованою гастроезофагеальною рефлюксною хворобою. Білок Клото і мітохондріальна супероксиддисмутаза досліджували в сироватці крові імуноферментним методом. Дослідження мітохондріальної супероксиддисмутази і білка Клото показало достовірне збільшення цих показників у пацієнтів з гастроезофагеальної рефлюксної хворобою і аутоімунним тиреоїдитом у порівнянні з контрольною групою і групою з ізольованою гастроезофагеальною рефлюксною хворобою. Білок Клото і мітохондріальна супероксиддисмутаза можуть використовуватися в якості біомаркерів прогресування гастроезофагеальної рефлюксної хвороби у молодих пацієнтів з супутнім аутоімунним тиреоїдитом.

Ключові слова: гастроезофагеальна рефлюксна хвороба, автоімунний тиреоїдит, білок Клото, мітохондріальна супероксиддисмутаза.

The study is a fragment of the research project "Mechanisms of gastroesophageal reflux disease formation with concomitant pathology and development of methods for its pathogenetic correction in students", state registration No. 0116U002441.

In recent years, the modern clinical picture of internal diseases has been characterized by an increase in the pool of chronic non-infectious pathology of internal organs, among which gastroesophageal reflux disease (GERD) and autoimmune thyroiditis (AIT) are discussed. The current prevalence of GERD in the western population is about 20%, while symptoms occur daily in about 7%, weekly – in 14%, and monthly – in 15-40% of the adult population [3]. Alarming is the fact that the clinical implications of GERD are increasingly manifested at a young age [11].

Mudyanadzo T. et al. found that changes in the esophagus at the molecular level occur even before the appearance of morphological changes in the mucous membrane [12]. This provides a basis for considering an alternative concept of the GERD pathogenesis, which is that esophageal reflux leads to inflammation of the esophagus not only due to irritation with acid content, but largely due to cytokine-induced mechanisms [2, 5].

Along with this, there is a hyperproduction of reactive oxygen species (ROS) by epithelial cells of the esophageal mucosa [6, 14]. The resulting oxidative stress initiates the triggering of regulatory mechanisms, which is manifested by the expression of enzymatic antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase, which constitute the main mechanisms of cellular defense against ROS [3]. In this case, mitochondrial, manganese-containing, superoxide dismutase (MnSOD) plays one of the main roles in the detoxification processes of ROS. Deviations of its functions or expression can lead to the development of various diseases as a result of oxidative stress and disruption of metabolic processes localized in mitochondria [7].

The Klotho protein inhibits the aging process and suppresses the activity of TNF-α, claims to be a mediator that mediates both anti-inflammatory and antioxidant effects. The participation of this protein in the

pathogenesis of an extremely wide range of diseases makes it possible to consider the Klotho protein as a potential participant in the pathogenesis of esophageal lesions [8].

Oxidative stress accompanies the course of thyroid diseases. It is believed that the mechanisms by which the intensification of lipid peroxidation processes is realized in two clinical states are different: increased production of ROS in hyperthyroidism and low availability of antioxidants in hypothyroidism [10]. Despite a significant amount of research on the GERD and AIT pathogenesis, the search for markers of the progression of nosologies with their comorbidity in young people remains relevant.

The purpose of the work was to study the levels of Klotho protein and mitochondrial superoxide dismutase in young patients with comorbid gastroesophageal reflux disease and autoimmune thyroiditis.

Materials and methods. 165 people were examined, among them 120 patients had 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 examined (75.56%) with isolated GERD were women, 27 (22.5%) and 11 (24.44%) respectively were men. Control group is consisted of 20 practically healthy individuals, all respects corresponded to the examined groups.

The study adhered to the 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. Written consent was obtained from each patient, 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.

Recommendation of the Montreal Consensus (2006), protocols for the management of patients with this nosology and ICD-10 were used for verification of the GERD diagnosis. Erosive and non-erosive forms of the disease were determined during esophagogastroduodenoscopy (EFGDS) ("Fuginon", Japan) according to the recommendations of the Los Angeles classification. A histomorphological study of the obtained biopsy material from the mucous membrane of the esophagus was carried out.

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

Klotho protein and MnSOD were studied in blood serum by enzyme immunoassay using a commercial test system («Elabscience», USA) on enzyme immunoassay analyzer Labline-90 (Austria) 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. The methods of nonparametric statistics were used: the Kruskal-Wallis test, the median test, the Mann-Whitney test, chi-squared (χ^2) test..

Results and discussion. The morphological form of the GERD was revealed during EFGDS. Erosive GERD was diagnosed in 34 patients (28.3%) of the main group and 11 patients (24.4%) of the comparison group. In other cases, patients had a non-erosive form of GERD: 86 (71.7%) and 34 (75.6%) cases respectively. The grades of erosive esophagitis were set, according to the Los Angeles classification (1994) (table 1).

Table 1

The distribution of various esophagitis degrees in the examined groups

Esophagitis degree	GERD+AIT (n=34)		GERD (n=11)		Significance of difference[1]
	N	%	n	%	
A	6	17.7	7	63.6	df=3 χ^2=8.772 p=0.033
B	18	52.9	3	27.3	
C	8	23.5	1	9.1	
D	2	5.9	0	0	

Note: [1] $p<0.05$ – the difference is statistically significant.

There were no statistically significant differences in the incidence of the GERD erosive form in the examined groups of patients (df=1, χ^2=0.250, p=0.618). However, a detailed analysis of the structure of the occurrence of various degrees of severity of erosive esophagitis showed that the course of GERD against the background of AIT is accompanied by a statistically significant redistribution towards the aggravation of the severity of the degree of the esophagus erosive lesion compared with isolated GERD (df=3, χ^2=8.772, p=0.033).

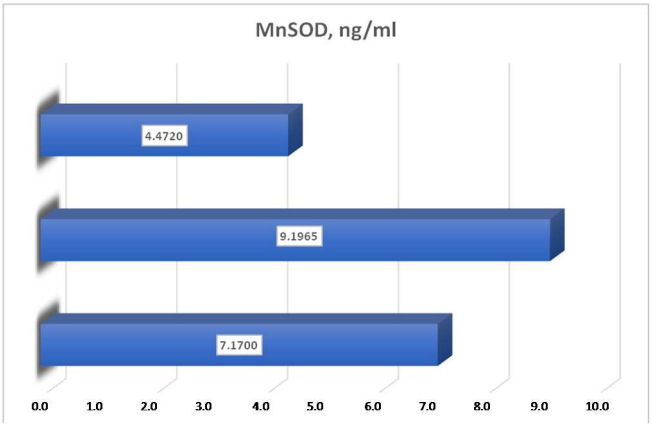

Fig. 1. Level of manganese superoxide dismutase in groups, ng/ml

The determination of MnSOD has shown an increase in this indicator in both study groups (Figure 1). The median and interprocentile range of total MnSOD activity in the group of patients with isolated GERD was 7.1700 (6.1056; 8.1948) ng/ml, which was significantly higher than in the group of somatically healthy individuals – 4.4720 (3.7010; 5.2325) ng/ml (U=279.5, p<0.01). This indicator was 9.1965 (7.2480; 11.6385) ng/ml in patients with a combined course of GERD and AIT; its increase was statistically significant, both in comparison with the control group (U=386, p<0.01) and the comparison group (U=108, p<0.01).

Thus, an increase in antioxidant defense activity was found in patients with both isolated GERD and in combination with AIT, as compared to the group of somatically healthy individuals. Apparently, the increased expression of MnSOD, which controls the more specific second phase of xenobiotic detoxification, can be explained by the failure of the total antioxidant defense, which reflects the first phase of the antioxidant system, which, due to the transition of physiological apoptosis to pathological one, does not ensure the neutralization of toxic substances.

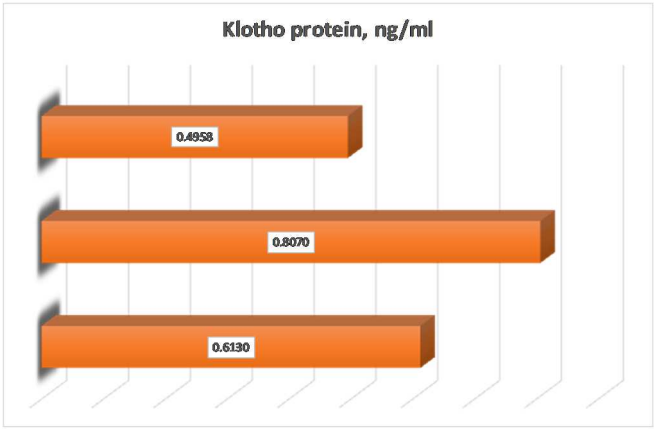

Fig. 2. Klotho's protein content in blood serum, ng/ml

In the study of the Klotho protein content in the blood serum, its increase was revealed in the examined groups in comparison with the control patients (fig. 2).

In patients with isolated GERD, the Klotho protein level was 0.6130 (0.4612; 0.7630) ng/ml, which was significantly higher than the control values (0.4958 (0.3679; 0.6098) ng/ml, U=279.5, p<0.01). With the GERD and AIT comorbidity, the increase in the studied value persisted (0.8070 (0.6110; 1.1840) ng/ml) and it was statistically significant both in relation to the norm (U=320.5, p<0.01) and in the group with isolated GERD (U=1570, p<0.01).

A direct correlation was established between the Klotho and MnSOD protein parameters, both in the combined course of GERD and AIT (r=0.724; p<0.05) and in the group with isolated GERD (r=0.683; p<0.05).

Analysis of the MnSOD and Klotho protein content, taking into account the morphological form of GERD, has shown that the erosions of the esophageal mucosa was not accompanied by significant changes in these indicators compared with the non-erosive form of the disease, both in the main group and in the comparison group. However, there was a trend towards a decrease in MnSOD levels in patients with erosive GERD in both groups (table 2).

Table 2

Content of MnSOD and Klotho protein depending on morphological form of GERD

Indicator	Group	Morphological form of GERD		Significance of difference[1]
		Erosive	Non-erosive	
MnSOD, ng/ml	Main group	8.3449 (6.488; 10.2433)	9.3458 (7.4743; 12.5117)	U=1112.5 p=0.079
	Comparison group	6.7666 (5.1572; 8.1946)	7.2828 (6.1068; 8.1946)	U=166 p=0.579
Klotho protein, ng/ml	Main group	0.7594 (0.5291; 1.2522)	0.8137 (0.6215; 1.1635)	U=1310.5 p=0.563
	Comparison group	0.6547 (0.4198; 0.7792)	0.5995 (0.5234; 0.7625)	U=179.5 p=0.843

Note: [1] p<0.05 – the difference is statistically significant

Detailed statistical analysis revealed a correlation between the levels of Klotho protein (r=0.688; p<0.05) and MnSOD (r=0.702; p<0.05) and the severity of erosive esophagitis in patients with isolated GERD. In the main group, these associations were stronger – (r=0.804; p<0.05) and (r=-0.867; p<0.05)

respectively, and the correlation with MnSOD was negative, which was probably a consequence of the significantly higher incidence of severe erosive lesions of the esophagus in patients of the main group.

The study revealed an increase in the level of MnSOD in patients with GERD in relation to those in the group of healthy individuals, which can be explained by the controlled antioxidant system reaction in response to an increase in the formation of lipid peroxidation products. In this case, the increased expression of MnSOD in patients with comorbid pathology can be explained by the presence of an additional autoimmune component under AIT conditions, in which the sequence and the adequacy of the immune response are disrupted. At the same time, the erosive lesion of the esophagus was associated with a decrease in MnSOD activity compared to the indices in the non-erosive form of GERD in both groups. Apparently, the intensification of inflammatory processes leading to the erosion of the esophageal mucosa increases the load on the antioxidant system, which leads to its gradual "depletion" and, as a consequence, to inadequate neutralization of superoxides.

Such a multidirectional expression of SOD can also be due to the duality of its effects, since, despite its high specificity, SOD can acquire the properties of a prooxidant by interacting with hydrogen peroxide and triggering the formation of a hydroxyl radical and superoxide anion under certain conditions. It should be noted that the cause of the initiation of the pathological process can be any deviations in the SOD expression: its inhibition is associated with insufficient ROS detoxification, while SOD hyperactivation leads to an intensification of the cytotoxic effect of hydrogen peroxide, which is formed as a result of dismutation of superoxide [1].

The synthesis of MnSOD increases not only due to an increase in the production of oxygen radicals as a stimulating factor, but also by activating the Klotho protein [9], which, in addition to anti-aging properties, has antioxidant and anti-inflammatory effects [4, 15]. The results of the study confirm this thesis: an increase in the Klotho protein level compared to the control group was recorded both in the group of patients with isolated GERD and in the comorbidity of GERD and AIT. The young age of the patients allowed us to move away from the anti-aging interpretation of changes in Klotho's protein content. Its hyperproduction was probably due to the "breakthrough" of the first line and the inclusion of the second phase of antioxidant defense – the Klotho-induced MnSOD, the protective function of which is to convert superoxides into hydrogen peroxide by hydrolysis with the subsequent formation of water [19, 13].

Conclusion

1. The comorbidity of GERD and AIT in young people is not accompanied by an increase in the incidence of the GERD erosive form; however, it is associated with a significant redistribution of the degree of erosive lesion to the esophagus in the direction of aggravation compared with the isolated course of the disease.

2. The level of MnSOD and the polyfunctional Klotho protein both in patients with a combined course of GERD and AIT and in patients with isolated GERD exceeds the values of the control group, while the concomitant AIT significantly aggravates the severity of these deviations.

3. The presence of the GERD erosive form is not accompanied by significant changes in the MnSOD and the Klotho protein values as compared to patients with the non-erosive form of the disease, both in the main group and in the comparison group. However, these indicators are correlated with the severity of the erosive lesion of the esophagus.

4. The Klotho protein and MnSOD can be used as biomarkers of GERD progression in young patients with concomitant AIT.

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N.A Zolotarova, O.V. Solomko
Odesa National Medical University, Odesa

SIDE EFFECTS CORRECTION IN COMBINED LIPID-CORRECTING THERAPY WITH STATINS AND FIBRATES USING LOW-MINERALIZED MINERAL WATER

e-mail: es7700@gmail.com

The side effects of the combination of statins and fibrates in patients with stable angina and low-mineralized silicon hydrocarbonate calcium-magnesium mineral water in their correction were studied. 64 patients were examined, divided into 2 groups. The first group received atorvastatin (10 mg) and fenofibrate (145 mg), the second group received low-mineralized silicon hydrocarbonate calcium-magnesium mineral water. It was found that use of atorvastatin and fenofibrate does not cause serious side effects, but undesirable effects, levels of alanine aminotransferase and creatine phosphokinase, increase. The positive effect of low-mineralized mineral water on clinical (prevention of symptoms from the digestive and hepatobiliary systems) and biochemical parameters were shown. Levels of creatine phosphokinase, alanine aminotransferase and aspartate aminotransferase decreased, significantly differing from group I ($p<0.05$). This allows us to recommend a low-mineralized silicon hydrocarbonate calcium-magnesium mineral water course for the prevention and treatment of side effects that occur at the beginning of statins and fibrates therapy.

Key words: side effects, statins, fibrates, mineral water, stable angina.

Н.А. Золотарьова, О.В. Соломко

КОРРЕКЦІЯ ПОБІЧНИХ ЕФЕКТІВ ПРИ КОМБІНОВАНІЙ ЛІПІДО-КОРЕГУЮЧІЙ ТЕРАПІЇ СТАТИНАМИ І ФІБРАТАМИ, ІЗ ЗАСТОСУВАННЯМ МАЛОМІНЕРАЛІЗОВАНОЇ МІНЕРАЛЬНОЇ ВОДИ

Вивчено побічні ефекти комбінації статинів і фібратів у хворих стабільною стенокардією і ефективність маломінералізованої кремнієвої гідрокарбонатної кальцієво-магнієвої мінеральної води в їх корекції. Обстежено 64 хворих, розділених на 2 групи, перша група отримувала аторвастатин (10 мг) і фенофібрат (145 мг), друга–додатково маломінералізовану кремнієву гідрокарбонатну кальцієво-магнієву мінеральну воду. Встановлено, що використання аторвастатину і фенофібрату не викликає серйозних побічних дій, але зростають небажані явища, рівні аланінамінотрансферази та креатинфосфокінази. Показано позитивний вплив маломінералізованої мінеральної води на клінічні (запобігання симптомів з боку травної та гепатобіліарної систем) та біохімічні показники. Знизились рівні креатинфосфокінази, аланінамінотрансферази і аспартатамінотрансферази, що значимо відрізнялось від І групи ($p<0,05$). Це дозволяє рекомендувати курсовий прийом маломінералізованої кремнієвої гідрокарбонатної кальцієво-магнієвої мінеральної води для профілактики і лікування побічних дій, що виникають на початку терапії статинами і фібратами.

Ключові слова: побічні дії, статини, фібрати, мінеральна вода, стабільна стенокардія.

The work is a fragment of the research project "The features of vascular disturbance in the patients of cardioreumatological profile: modern methods of diagnostics and therapy", state registration No. 0119U003576.

Diseases of the circulatory system consistently occupy a leading position in the structure of total mortality [1, 10]. Traditionally, the first place is occupied by coronary heart disease (CHD), whose share in the total mortality structure has increased from 66.6% (2005) to 68.9% (2015 [1].

It is known that one of the key factors in CHD progression is dyslipidemia. First-line hypolipidemic drugs are statins, however, many patients do not receive adequate therapy, due to poor adherence to taking