ACTIVITY OF MITOCHONDRIAL ANTIOXIDANT DEFENSE SYSTEM IN YOUNG PATIENTS WITH GASTROESOPHAGEAL REFLUX DISEASE

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Abstract

Background. Despite numerous studies, the pathogenesis of gastroesophageal reflux disease remains unclear. **Aim of research:** assessment the activity of antioxidant defense system in young patients with GERD based on expression of biomarker associated with mitochondrial function. **Material and methods.** The study included 45 patients with gastroesophageal reflux disease. The examined contingent was presented by students age from 18 to 25 years. 20 healthy persons were included as control group. Levels of manganese superoxide dismutase were determined in blood serum of study persons with enzyme immunoassays (ELISA, Elabscience, USA). Statistical data processing by the Statistica Basic Academic 13 for Windows En local was made. **Results.** Gastroesophageal reflux disease in young patients is characterized by significantly increasing of manganese superoxide dismutase as compare to control group (7.1700 ng/ml vs 4.4720 ng/ml respectively, $p<0.01$). Presence of erosion in esophagus mucous doesn't accompanied by significant changes of evaluated parameter as compare with non-erosion form of disease in patients. **Conclusion.** The elevation in young patients with GERD the biomarker of mitohondrial antioxidant defense system we may speculate as adaptive response contributing to non-specific citoprotection. Taking to account the publishing facts about dual role of manganese superoxide dismutase it is necessary to monitoring antioxidant enzyme in patients with gastroesophageal reflux disease for prediction of possible complications and outcome.

Keywords: *gastroesophageal reflux disease, young age, manganese superoxide dismutase.*

1. Introduction

Gastroesophageal reflux disease (GERD) is classified as chronic acid reflux [1]. According to prospective population-based cohort study, the prevalence of GERD is 2% to 25 % [2]. Symptoms of GERD are experienced in a range from mild to severity in a form of Barrett's esophagus [3]. Pathogenesis of GERD includes multifactorial mechanism firstly acid and acid-pepsin which considered as injury factors for esophageal squamous epithelium [4]. According to the findings based on animal models of chronic acid exposure and histological examination of biopsy material obtained in patients by esophagoscopy, esophageal squamous cells the inflammatory mediators have been revealed. The new theory of pathogenesis esophageal inflammation related to relationship between cytokine profile and esophageal inflammation was proposed [5].

Inflammation induced by cytokines results in overproduction of reactive oxygen species (ROS). The main source of superoxide radicals in the cell are mitochondria, the place for oxygen metabolism. It is known that a lot of diseases of internal organs are accompanied by formation of oxidative stress with damage to biological macromolecules and cell membranes [6]. Oxidative stress is the considered as imbalance between components of pro- and antioxidants systems which have been formed in evolutionary process. In order to block the harmful effect of oxidative stress in case of ROS increase, activation of the antioxidant defense system occurs.

Nonenzymatic scavenger of antioxidant defense are enzymes superoxide dismutases which are the major ROS detoxifying enzymes of the cell and catalyze dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen. Superoxide dismutases are metalloenzymes and hence, require a metal cofactor for their

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activity. The mitochondrial matrix stores manganese superoxide dismutase (MnSOD/SOD2), also called super dismutase manganese or mitochondrial SOD2, the products of the electron transport chain. MnSOD is an integral mitochondrial protein known as a first line antioxidant defense against superoxide radical anions [7, 8]. In the analyzed literature we did not find the data relevant to state of mitochondrial antioxidant defense system in patients with GERD.

2. Purposes, subjects and methods:

2.1 Purpose. Assessment the activity of antioxidant defense system in young patients with GERD based on expression of biomarker associated with mitochondrial function.

2.2. Subjects & Methods. This study involved 45 patients with GERD. The examined group consisted of university students aged from 18 to 25 years, median age was 21.2±2.4 years. 34 patients (75.6%) with GERD were women, and 11 (24.4%) men. The history of GERD did not exceed 3 years. 20 healthy age-matched persons were chosen as a control group.

The study was conducted within the period of 2017–2019 in the inpatient hospital of Department of General Practice-Family Medicine and Internal Diseases of Kharkiv National Medical University (Ukraine) and in the inpatient hospital of Department of Therapy, Rheumatology and Clinical Pharmacology of Kharkiv Medical Academy of Postgraduate Education (Ukraine). GERD diagnosis was verified according to the recommendation of the Montreal Consensus (2006), protocols for the management of patients with GERD. The morphological form of the disease was revealed during esophagogastro-duodenoscopy ("Fuginon" system) 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.

Levels of MnSOD were determined in blood serum with enzyme immunoassays (ELISA, Elabscience, USA). Statistical data processing by the Statistica Basic Academic 13 for Windows En local was made for comparison of categorical variables.

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, the 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.

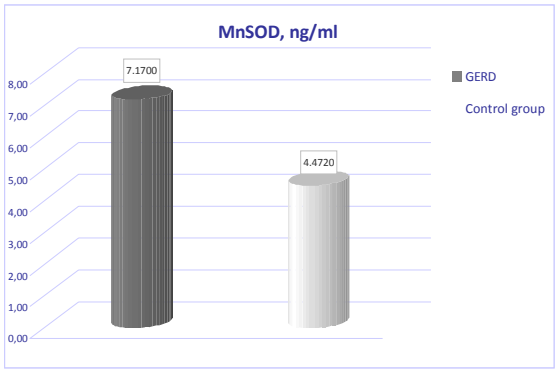
Conflict of interests

There is no conflict of interests.

3. Results and discussion

The morphological form of GERD was revealed during EFGDS. Erosive GERD was diagnosed in 11 patients (24.4 %), in other cases, study participants had a non-erosive form of GERD – 34 (75.6 %) patients. According to the Los Angeles classification (1994) 7 patients had grade A of erosive esophagitis, 3 examined – B and grade C was registered at 1 patient.

The levels of MnSOD in blood serum of the study patients and controls are presented in *Figure*.



Levels of MnSOD in individuals of study and control groups

The patients had significantly higher serum levels of MnSOD compared to the control persons. Median and interpercentil deviation of MnSOD was 7.1700 (6.1056; 8.1948) ng/ml in patients with GERB and significantly higher compared to control persons – 4.4720 (3.7010; 5.2325) ng/ml ($U = 513$, $p<0.01$).

We evaluated MnSOD plasma levels in patients with GERD depending to morphological forms of the damage to the esophagus mucous assessed by a histomorphological study of the obtained biopsy material. The results are shown in *Table*.

Presence of erosion in esophagus mucous was not accompanied by significant change of evaluated parameter as compared with non-erosion form of disease in patients. Nevertheless the tendency to decreasing MnSOD plasma levels in patients with erosion form GERD was found. The explanation of this facts related to the data

Levels of MnSOD in patients depending on damage of the esophageal mucous

Parameter	Erosive morphological form of GERD	Non-erosive morphological form of GERD	Statistical significance between groups
MnSOD, ng/ml	6.7666 (5.1572; 8.1946)	7.2828 (6.1068; 8.1946)	U=347, p>0.05

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

that underline that differ stressors (chloric acid, nonsteroid antiinfammatory drugs and others) generate free radicals which not only injure epithelial cells, but also cause free radical accumulation in the mitochondria leading to organ damage, and functiona changes accompanied by cell apoptosis and death [9].

In order to prevent oxidative stress, the cell must respond to ROS by mounting an antioxidant defense system. MnSOD has long been recognized to be important against mitochondria-generated oxidants because of its well-known superoxide dismutase activity. Indeed, several studies have established that many of the cellular effects of MnSOD can be attributed to the superoxide scavenging ability of the enzyme. MnSOD in the mitochondria converts highly toxic superoxide O_2 into less toxic hydrogen peroxides H_2O_2 [10]. We found elevation of activity of antioxidant enzyme associated with mitochondria in patients with GERD. We may speculate that young patients with GERD according to their age have preserved beneficial capacities of MnSOD to act as a superoxide dismutase, impact on behavioral harmful xenobiotics and protect mitochondria against oxidative damage. This effect is less pronounced in patients with erosion form of GERD.

Taking to account the results of some studies it can be suggested that increased expression of MnSOD may be useful by regulating the mitochondrial redox status, prevent the cells apoptosis and protect of some organs [11]. The opposite situation occurs when a decreased expression of MnSOD resulted in deleterious effects.

Nevertheless, further studies have shown that MnSOD plays multiple roles in cells beyond its proposed antioxidant functions. Ansenberger-Fricano K., da Silva Ganini D., Mao Mao. et al. (2013) in experimental procedure on recombinant MnSOD from human mitochondria cells treated with glucose oxidase or exogenous H2O2 using electron paramagnetic resonance, visible spectrometry studies, gel electrophoresis and Western Blot Analysis, fluorescent Immunocytochemistry, gene-specific quantitative PCR to assay mitochondrial DNA (mtDNA) found that in the presence of H2O2 level of overexpression MnSOD can possesses peroxidase activity, leads to mitochondrial protein oxidation, predisposes mitochondria to oxidative stress, sensitizes mtDNA to oxidative damage [12]. The authors summarized that overexpressed MnSOD may gain the function as a peroxidase contributes to mitochondrial dysfunction. Moreover epidemiologic and clinical studies indicated a conflicting role of SOD_2 gene in the production and elimination of H_2O_2 that can be considered a protective antioxidant, as well as a pro-oxidant driving cancer [13].

It was identified that a higher expression of SOD_2 in human esophageal squamous cell carcinoma samples was associated with TNF ? expression and poor overall survival in patients with cancer, suggesting that SOD_2 may act as an oncogene [14]. The expression of SOD_2 in breast cancer is significantly correlated with TNM stage and axillary lymph node metastasis. SOD_2 may affect the proliferation, invasion and metastasis of breast cancer cells [15].

The recent facts gave comprehensive explanation of activity of MnSOD to hypothesize that mild (2–3 fold) MnSOD expression effectively reduces mitochondrial ROS generally correlating with improved mitochondrial function whereas diminishment or overexpression of MnSOD in mitochondria result in oxidative stress and damage [12]. According to this new interpretation of MnSOD role we should provide the control of levels of antioxidant enzymes in patients with GERD relevant to symptoms and signs, results of esophagoscopy in order to monitor the clinical features and treatment efficacy.

4. Conclusion

The present study demonstrates that patients with gastroesophageal reflux disease have a significant increase in manganese superoxide dismutase levels as compared to the persons from the control group that reflects in case of mucosa esophageal inflammation the compensatory reaction of intracellular enzyme directed to tissue protection. Taking to account the published facts about the dual role of manganese superoxide dismutase, it is necessary to monitor antioxidant enzyme in patients with gastroesophageal reflux disease for prediction of possible complications and outcome.

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