ANALYSIS OF ANTIOXIDANT PROTECTION INDICATORS FROM THE POSITION OF AGE CHANGES IN CHRONIC PANCREATITIS PATIENTS

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Abstract

Introduction. The identification and assessment of all external and internal factors affecting the development of the chronic pancreatitis (CP) is one of the most important conditions for the personalized selection of its adequate therapy. The authors analyze the indicators of lipid peroxidation (LPO), endogenous intoxication (EI) and the antioxidant defense system (AODS) in CP patients of different ages.

The objective: to assess the indicators of LPO, EI and the AOS system in CP in different year classes.

Materials and methods. 247 CP patients were examined; they were divided into 3 groups depending on age. The control group consisted of 30 apparently healthy persons of different ages without clinical signs of CP. LPO was assessed by the level of malonic aldehyde (MA) according to the method of V. B. Gavrilov et al. AODS was determined by the level of SOD; ceruleoplasmin (CP) was determined in blood serum (according to Ravin); free glutathione (SH-groups) was determined by Boyer method with sodium p-mercurbenzoate; catalase - according to the level of decomposition of hydrogen peroxide and determination of the ratio of catalase number to the number of erythrocytes. Statistical processing of the results were carried out with the use of standard software packages Microsoft Excel and Statistica 6.0. The results were evaluated according to Student's t - criteria.

Results: It was revealed that LPO activation in terms of MDA, MM and EI response significantly increases in the middle age category (p <0.05) and in CP patients over 66 years old (p <0.001). Simultaneously, weakening of enzymatic (by levels of SOD, catalase) and non-enzymatic (according to the state of free glutathione, retinol and tocopherol) composition of the AODS (p <0.05) took place. It was established that with the increase of biological age depletion of host defenses followed by signs of oxidative stress and endotoxemia took place. It was proved by SOD (p <0.05) and (p<0.001) and catalase (p <0.001) decrease in middle-aged and elderly CP patients. The study of the vitamin balance showed a decrease in retinol and tocopherol levels in older age CP patients, in the group of elderly patients their levels were below normal (p <0.001).

Conclusions. The results obtained will make it possible to create a scheme of differentiated approach to clinical examination of CP patients depending on their age, and predict of polyinutrient disorders accurately and timely.All human studies were conducted in compliance with the rules of the Helsinki Declaration of the World Medical Association "Ethical principles of medical research with human participation as an object of study". Informed consent was obtained from all participants.

Keywords: *chronic pancreatitis, patients of different ages, antioxidant system, lipid peroxidation, endotoxicosis*

Introduction

Of all gastrointestinal tract diseases, chronic pancreatitis (CP) is perhaps the most questionable, as it is polyetiological and polymorbid pathology, which is characterized by inflammatory and degenerative changes in the pancreas (PG). It often has often severe and recurrent course and results in a number of complications [2, 3].

According to WHO age classification (2015), the following age periods are distinguished: from 25 to 45 years - young age, 46 - 65 years - average, 66-75 years - elderly, 75 - 90 years - senile and after 90 years - the age of long-lived. According to the current WHO classification, old age begins at the age of 65 years old. Attempts to divide humans by age groups are to some extent determined by the average life expectancy, changes in which directly depend on the age. The most significant changes that indicate aging are observed in middle age and are associated with physiological characteristics of the body [8, 9, 13].

In Ukraine, the average life expectancy is 73 years for women and 58 years for men. It is significant that the process of increasing the number of elderly and senile people is being observed all over the world. According to UN forecasts, by 2025 the number of people over 60 will exceed 600 million and account for more than 15% of the planet adult population [9, 11].

Today there is a clear increase in the prevalence of PG pathology in young people of working age, but the exacerbation of the chronic process in the case of definite conditions occurs in all age groups. The manifestation of the inflammatory process in the pancreas begins with a damaging effect on the gland of one or a combination of several etiological factors. This also needs to be taken into account when studying the age characteristics of CP.

It should be emphasized that in the presence of exogenous and endogenous destabilizing factors, which often leads to diseases of the gastrointestinal tract, acute or chronic stress develops [1]. One of the manifestations of the strength and duration of stress is the activation of free radical oxidation of unsaturated fatty acids, which directly regulate the physicochemical processes occurring at the cellular and subcellular levels. Under physiological conditions, in the absence of pathology, the antioxidant system (AOS) protects the integrity of cell membranes from the destructive effects of oxidative reactions and controls the processes of lipid peroxidation (LPO) and proteins [7, 10]. The age trend of these processes in CP requires in-depth study, which motivated the study

Objective: to investigate the indicators of lipid peroxidation, endogenous intoxication and antioxidant defense system in chronic pancreatitis in the age aspect.

Methods

247 CP patients of different age were examined. "Ternopil City Municipal Hospital №2" and "Odessa Regional Clinical Medical Center " were used as clinical sites. The object of the study were "Medical card of an outpatient" and "Medical card of an inpatient". The examination was done in 2014 - 2020. The diagnosis of "chronic pancreatitis" was established on the basis of the clinical protocol adopted in accordance with the Order № 638 of the Ministry of Health of Ukraine dated 10.09.2014 [5].

Criteria for inclusion in the study: patients aged 21 - 75 y.o., diagnosed with CP without exacerbation (often incomplete remission), mean age - (53.2 ± 1.4) years; men - 133 (53.8%), women - 114 (46.2%).

Exclusion criteria: blood diseases and oncological processes, acute infectious diseases during the last 3 months, exacerbation of chronic pathology, state of decompensation of vital organs, pregnancy.

Depending on the biological age CP patients were randomized into 3 groups: I - 45 years old, II – 46 - 65 years old and III group consisted of patients over 66.

The control group consisted of 30 apparently healthy persons of different ages without clinical signs of CP, comparable in age, sex and social status.

Evaluation of LPO was performed by the level of malonic aldehyde (MA). It was determined by reaction with thiobarbituric acid by the method of V. B. Gavrilov et al. [4]. AOS parameters were determined by the content of superoxide dismutase (SOD) in blood. SOD was estimated by the ability to compete with nitrotetrazolium blue for superoxide anions. The content of ceruloplasmin (CPN) was determined by H. A. Ravin (1956). The pool of free glutathione (SH-group) was determined by Boyer P. D. (1954) method with sodium p-mercurbenzoate. The content of catalase was determined by the cleavage of hydrogen peroxide and the ratio of the

catalase number to the number of erythrocytes (million in 1 µl of blood). The content of retinol in blood plasma was determined by hydrolysis and extraction from plasma using organic solvents and subsequent spectrophotometry. Determination of tocopherol was done by oxidation of tocopherols with ferric chloride and determination of ferrous iron with subsequent complexation with 2 - 2 dipyridyl. Evaluation of endogenous intoxication (EI) was performed on the content of medium weight molecules (MWM1 and MWM2) by the method of Gabrielyan N. I. [13] (1985). According to the method mentioned the determination of optical densities of serum released from high molecular weight proteins and lipids at wavelengths of 254 and 280 nm was made. The EI index was evaluated by the sorption capacity of erythrocytes [6]. Statistical processing of the results was performed with the use of standard packages Microsoft Excel and Statistica 6.0 and evaluated according to Student's criteria (t-test).

Results

Analysis of LPO, namely unsaturated fatty acids, endotoxicosis and AOS in patients with CP of different ages is presented in Table 1.

The significant activation of LPO by MA in CP patients was found, as well as strengthening of EI by MWM and EI index levels, weakening of enzymatic (according to the levels of SOD, catalase) and non - enzymatic (according to the pool of free glutathione, retinol and tocopherol) AOD. Significant increase CPN in the general group in relation to the control group, in our opinion, is due to the presence of insignificant activity of the inflammatory process in CP patients, even without exacerbation.

Table 2 shows the parameters of POL-AOS and EI in different age groups of CP patients. The increase of oxidative stress phenomena in CP with increasing biological age has been established. With varying degrees of reliability, this provision has been proven for indicators of MA, MSM and IE index, which showed the activation of the LPO and the accumulation of toxic products in CP patients, even outside the exacerbation, which progresses with age. At the same time, with increasing biological age of CP patients there is a decrease in AOS indexes. It should be noted that a probable decrease in CP patients of older age (over 66 y.o.) against the background of a general increase in this indicator in CP. The establishment of this trend allows us to conclude about the depletion of the body's defense mechanisms with increasing phenomena of oxidative stress and endotoxicosis, which increases with age.

The study of vitamin balance showed varying degrees of reliability of vitamin levels decrease in groups of older age CP patients. In the group of elderly patients the levels of vitamins were lower than normal, so in this age group the significant hypovitaminosis of retinol and tocopherol was identified. Thus, a decrease of tocopherol and retinol content with CP patients aging indicates a decrease in the vitamin status of aged patients and depletion of the AOS system, which is a pathogenetic factor in CP progression.

The established facts will allow to form a differentiated approach to the management of different age groups CP patients, as well as to offer differentiated programs of complex treatment of the patients under study into account the age trend of EI, LPO-AOS.

Conclusions

1. The presence of significant activation of lipid peroxidation by MA, increased endogenous intoxication by MSM and IEI, as well as attenuation of enzymatic (by SOD and catalase in blood serum) and non-enzymatic (by free glutathione, retinol and tocopherol) parts of the antioxidant defense system ($p < 0.05$), which statistically significant deepen with an increase of biological age.

2. Activation of ceruloplasmin in the younger and middle age groups ($p < 0.05$) in relation to the control group and decrease of its activity in CP patients aged over 66 years ($p < 0.001$) was established. This proved a decrease in the protective potential of the ceruloplasmin system in the patients of older age group.

3. Decreased tocopherol and retinol levels in the older age group of CP patients reached the level of hypovitaminosis of these antioxidant vitamins and proved the presence of trophological vitamin deficiency, which progresses with age. This is one of the factors reducing the functional capacity of the pancreas with aging.

In the perspective of further research the authors are going to propose and scientifically substantiate differentiated programs of complex treatment of CP taking into account the patients' age.

Acknowledgments

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Table 1. The state of POL-AOS and endotoxicosis in chronic pancreatitis, n = 247

Parameters EI, POL-AOC	Comparison group	
	Control group, n=30	Chronic pancreatitis patients, n = 247
MWM1, relative units	332.10±2.63	476.41±7.32*
MWM2, relative units	149.50±1.22	228.51±2.96*
IEI, %	28.23±1.21	54,35±0.98*
MA, mmol/l	2.798±0.094	5.221±0.099*
SH-Group, mmol/l	71.20±2.13	60.93±1.34*
SOD, relative units	64.21±2.85	43.35±0.68*
Catalase, %	18.38±0.87	14.46±0.99*
CPN, mg/l	239.60±2.60	319.26±6.62*
Tocopherol, mcmol/l	114.05±0.35	62.74±1.73*
Retinol, mcmol/l	1.64±0.02	0.71±0.01*
Note: * - significant probability of change compared with the control group		

Table 2. Age trend of EI, POL-AOS parameters in chronic pancreatitis patients

EI, LPO, AOS	Control group, n=30	CP patients of different age, n = 247		
		Up to 45 y.o, n=83	46 - 65 y.o., n =86	Over 66 y.o., n=78
MWM1, relative units, 254 nm	332.10±2.63	448.92±13.04*	569.91±8.47** p<0,001	642.40±12.76** p<0,001
MWM1, relative units, 280 nm	149.50±1.22	176.81±5.12*	224.66±2.95** p<0,001	261.59±3.12** p<0,001
IEI, %	28.23±1.21	43.11±0.96*	57.71±1.58** p<0,05	64.16±1.86** p<0,01
MDA, mmol/l	2.798±0.094	4.68±0.07*	5.73±0.06** p<0,05	6.34±0.09** p<0,001
SH-group, mmol/l	71.20±2.13	63.34±0.21*	54.67±0.52** p<0,001	51.58±0.66** p<0,05
SOD, relative units	64.21±2.85	52.76±1.12*	44.22±0.58** p<0,05	36.41±1.13** p<0,001
Catalase, %	18.38±0.87	17.47±1.96	15.03±1.06*	12.89±1.14*
CPN, mg/l	239.60±2.60	347.76±8.89*	312.45±2.19** p<0,001	296.74±2.03** p<0,001
Tocopherol, mmol/l	114.05±0.35	71.26±3.56*	56.42±1.34** p<0,001	39.09±2.11** p<0,001
Notes: * - significance of differences in relation to the control group (p <0.001); ** - significance of differences in patients with CP up to 45 years (p <0.001); p – the significance of differences in relation to the previous group of patients				