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PECULIARITIES OF SYSTEMIC INFLAMMATION OF LOW INTENSITY IN PATIENTS WITH STABLE CORONARY HEART DISEASE CONCURRENT WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Ішемічна хвороба серця та неалкогольна жирова хвороба печінки є актуальними проблемами сьогодення в Україні та світі. Функціональні розлади роботи печінки потенціюють розвиток та прогресування ішемічної хвороби серця. Пусковим процесом атеросклерозу (АС) є хронічне системне запалення низької інтенсивності. Цей погляд почав активно формуватися останні два десятиріччя. Метою роботи було вивчення особливості системного запалення низької інтенсивності у хворих на стабільну ішемічну хворобу серця у поєднанні з неалкогольною жировою хворобою печінки. До дослідження були залучені 135 осіб хворих на ІХС: стенокардію напруги стабільну, I-II ФК, СН 0-I у поєднанні з неалкогольною жировою хворобою печінки та 30 здорових осіб. З метою дослідження рівня активності хронічного системного запалення низької інтенсивності у хворих визначали у крові рівень цитокінів -TNFα та IL-10, вміст реактанту гострої фази та фактору коагуляції — ФГ, маркер ендотеліальної дисфункції — кількість циркулюючих ендотеліальних мікрочасточок (ЦЕМ) CD32⁺ CD40⁺ та рівень експресії гену IκBα NF-κB у мононуклеарах периферичної крові. Дослідження рівню експресії гену mRNA IκBα у мононуклеарах крові, що відображає рівень транскрипційної активності NF-κB, у хворих на стабільну ІХС та ІХС у поєднанні з НАЖХП показало достовірне підвищення показника експресія гену mRNA IκBα на 88,5 % порівняно з хворими лише на стабільну ІХС. Аналіз функціонального стану ендотелію за допомогою ЦЕМ CD32⁺ CD40⁺ показав наявність ендотеліальної дисфункції у групах хворих на ІХС та ІХС в умовах НАЖХП. При порівнянні показників системного запалення низької інтенсивності та маркера ендотеліальної дисфункції у хворих на ІХС та ІХС у поєднанні з НАЖХП виявлено достовірне підвищення рівня TNFα, реактанта гострої фази та фактора коагуляції — ФГ та експресії гену mRNA IκBα у хворих на коморбідну патологію, що свідчить про підвищення рівню системного запалення низької інтенсивності у хворих на ІХС в умовах НАЖХП порівняно з групою хворих на ІХС.

Ключові слова: ішемічна хвороба серця, неалкогольна жирова хвороба печінки, хронічне системне запалення, ендотеліальна дисфункція.

Nowadays, coronary heart disease and non-alcoholic fatty liver disease are significant problems in Ukraine and world. Functional liver disorders potentiate the development and progression of CHD. The initiation process of atherosclerosis is a chronic systemic inflammation of low intensity. This view on atherosclerosis development has been forming during the past two decades. The aim of the research was to study the features/characteristics of systemic inflammation of low intensity in patients with coronary heart disease in combination with non-alcoholic fatty liver disease. The research involved 135 people with CHD: stable angina, I-II functional class, 0-I heart failure in combination with non-alcoholic fatty liver disease and 30 healthy individuals. We examined patients in terms of blood levels of cytokines -TNFα and IL-10, the content of the acute phase reactant and the coagulation factor, the marker of endothelial dysfunction is the amount of circulating endothelial microparticles (CEM) CD32⁺ CD40⁺ and the expression level of IκBα gene NF-κB in mononuclear peripheral blood. We studied the level of expression of the mRNA gene of IκBα in mononuclear cells, which reflects the level of transcriptional activity of NF-κB in patients with stable coronary artery disease and CHD in combination with NAFLD showed a significant increase in the expression of the mRNA gene of IκBα by 88.5% compared to patients with stable stable coronary heart disease. The analysis of the functional state of the endothelium with help of CEM CD32⁺

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CD40+ has shown the presence of endothelial dysfunction in the groups of patients with CHD and CHD in combination with of NAFLD. Comparison of the indicators of systemic inflammation of low intensity and marker of endothelial dysfunction in patients with CHD in combination with NAFLD revealed a significant increase of TNF α , acute phase reactant and coagulation fibrinogen factor and expression of the mRNA Ikb α gene in patients with comorbidity, indicating an increase the level of systemic inflammation of low intensity in patients with CHD in combination with NAFLD as compared with the group of patients with CHD.

Key words: coronary heart disease, non-alcoholic fatty liver disease, chronic systemic inflammation, endothelial dysfunction.

Introduction

This work is a part of planned research project of the Department of Propaedeutics of Internal Medicine with Medical Attendance, General Practice (Family Medicine), UMSA entitled: "Features of the course and prognosis of metabolic syndrome taking into account the genetic, age, gender aspects of patients, the availability of various components in metabolic syndrome and specific accompanying pathology and the ways of correction of detected disorders" (state registration No. 0114U001909).

Coronary heart disease and non-alcoholic fatty liver disease are relevant problems of the present day in Ukraine and in the world.

The initiation process of atherosclerosis is a chronic systemic inflammation of low intensity. This view on the development of atherosclerosis began active development last two decades [9,10]. Numerous studies prove the close connection between systemic inflammation of low intensity, oxidative stress and atherosclerotic lesions of the vascular wall [12].

Scientific achievements of recent years recognize endothelial dysfunction as one of the most important links in the pathogenesis of atherosclerosis [1,2,17]. Disturbance of the endothelium properties in result of damaging of the cell membrane by free radicals, modified LDL, antigenic complexes, monocytes-macrophages, cytokines, leads to activation of endothelial cells with subsequent apoptosis and formation of a stable imbalance of all endothelial-dependent functions [18].

Immunocompetent cells and cytokines play an important role in the formation of atherosclerosis.

The proliferation cytokines Th1 of immune response are IL-1, IL-6, IL-8, TNF- α , γ -interferon (IFN γ) have a wide range of effects at different levels of inflammatory process in the body as induces the synthesis of acute phase proteins by hepatocytes, involved in proliferation and differentiation of T- and B-lymphocytes, production of immunoglobulins, natural killers (NK), increase the functional activity of neutrophils, fibroblasts, activate phagocytosis, affect the expression of endothelial cells of adhesion molecules, etc. [3].

A powerful source of inflammatory cytokines is an excess of adipose tissue in the body. Adipocytes accumulated with lipids produce a large number of proinflammatory cytokines which are activators of chronic inflammation. The excess of fatty acids (FA) in the blood is also an important component of low-density heart disease.

The main component of chronic systemic inflammation (CSI) is the nuclear factor of Kappa B (NF-kB) [4,5,6,7,11], whose activation occurs with TNF α , IL-6.

The aim of the research is to study the features of systemic inflammation of low intensity in patients with stable coronary heart disease in combination with non-alcoholic fatty liver disease.

Materials and methods

In the research were involved 135 people with CHD: stable angina, I-II functional class, 0-I heart failure in

combination with non-alcoholic fatty liver disease and 30 healthy individuals.

The diagnosis of CHD was carried out according to the recommendations of the European Society of Cardiology, the Order of the Ministry of Health of Ukraine No. 436 dated July 3, 2006, "On approval of the protocols for the provision of medical care in the specialty" Cardiology", according to the Unified clinical protocol for medical care" Coronary heart disease: stable angina. Primary Aid", according to the Order of the Ministry of Health of Ukraine No. 816 dated 23.11.2011, and the Unified Clinical Protocol for primary, secondary (specialized) and tertiary (highly specialized) medical care" Stable coronary heart disease ", effective from 2015 [15].

Diagnosis of NAFLD was established in accordance with the recommendations of the Order of the Ministry of Health of Ukraine dated November 6, 2014, No. 826 "On Approval and Implementation of Medical-Technological Documents for the Standardization of Medical Assistance in Chronic Non-Infectious Hepatitis", as well as practical recommendations of the American Association for the Study of Liver Diseases, the American College of Gastroenterology and the American gastroenterological association [16,14].

The criteria of exclusion were the following : the cardiovascular system - the presence of hypertension above the second stage, unstable angina or angina III and IV classes, chronic heart failure (CHF) above stage I, acute myocardial infarction, congenital and acquired heart defects, diabetes mellitus type I, chronic and acute viral hepatitis, alcoholic liver disease, autoimmune hepatitis, Wilson-Konovalov's disease, hemochromatosis, rheumatic diseases, anemia, endocrine diseases, chronic liver and kidney disease with a disease deficiency I musculoskeletal inflammatory genesis in the acute stage, cancer.

In order to estimate the level of CSI measured concentration in serum cytokines: TNF α , IL-6 and IL-10 by (ELISA) which is based on solid-phase "sandwich" variant of enzyme immunoassay using mono- and polyclonal antibodies [13]. We used PCR to determine gene expression inhibitor of kappa B α (Ikb α) in real time (Real-time PCR) in peripheral blood mononuclear cells using detective amplifier DT Light ("DNA Technology", Russia). For cDNA, an inverse transcription reaction was performed using a set of reagents (SYNTOL, Russia). The total RNA was taken from a biological sample using a kit of reagents "fish-in-sol" (AmpliSens, Russia). The genome of the "household" GAPDH was used as a reference gene. The Ct method was used to analyze the data obtained and calculated using the formula $2^{-\Delta Ct}$ and $2^{-\Delta\Delta Ct}$. In order to establish endothelial dysfunction (ED) using flow cytometry laboratory method to determine the number TSEM in peripheral blood by identifying the expression of antigens CD32 and CD40 circulating endothelial microparticles (CEM) [8]. In the supernatant was determined by CD32+CD40+ - microparticles in flow cytometry «EPIX LX-MCL» (Beckman Coulter, USA) using the «System II TM software». Fluorescence was reached by argon lasers. Identification of endothelial phenotype was performed using

mouse monoclonal antibody (IgG) to surface antigens that have been labeled with fluorescent dyes. For control, fluorescence dye spotted with mouse IgG was used.

The statistical analysis of the data from the research results was carried out by the KyPlot[®] licensing program (KyensLab Inc., version 5.0). The hypothesis of normal distribution was checked by the criterion of Shapiro–Wilk.

The verification of the one set samples was carried out by the rank criterion of Crackel-Wallis. The Student's t-test was used for comparative analysis of the examination's results of the patients before and after treatment, the non-parametric pairwise criterion of Wilcoxon was used in the case of uneven distribution.

The data of three independent groups were analyzed with help odd Student's t-test and corrected by Bonferoniuum with normal distribution and for abnormal distribution the Still-Dwass criterion was used. The correlation between the variables was established by the Pearson's correlation analysis, in the case of non-uniform was used Spearman rank correlations. The results of the statistical analysis are presented in the form $X + \sigma$, where X means value, σ means square deviation. The data were evaluated as reliable when the significance level of p was <0.05.

The results and discussion

The goal of research of the activity level of chronic systemic inflammation of low intensity in patients with stable angina of CHD and CHD in combination with NAFLD were examined the blood levels of TNF α and IL-10 cytokines in the blood, the content of the acute phase reactant and coagulation factor - fibrinogen, the marker of endothelial dysfunction - the number of circulating endothelial microparticles CD32+ CD40+ and the level of expression of the I κ B α gene NF-kB in mononuclear peripheral blood.

The analysis of the level of cytokines revealed in both groups the study of increase in the concentration of proinflammatory cytokines - TNF α (in healthy subjects - 0.5 (percentile interval - 0-6) pg / ml), as well as an increase in the mean value of the anti-inflammatory IL-10 CC, which healthy individuals is 5 pg / ml. A significantly higher TNF α level in patients with coronary artery disease in combination with NAFLD (p <0.001) was found, compared with a group of patients with coronary artery disease. The probable difference between the groups for the level of IL-10 is not defined (p>0.05) (Table 1).

Table 1.
Levels of blood cytokines in patients with coronary artery disease in association with NAFLD

Group / Indicator, $X \pm \sigma$	Patients with CHD, n=25	Patients with CHD in combination with NAFLD, n=110
TNF α , pg / ml	8.48 \pm 2.15	10.56 \pm 3.74*
IL-10, pg / ml	10.64 \pm 3.54	11.43 \pm 2.12

Note* – the probable difference as compared with the data of patients with CHD (p <0,05).

The content of fibrinogen in the blood plasma was elevated in 37% of patients with stable angina CHD and in 54% of patients with CHD in association with NAFLD and a probable increase in patients with comorbidity was found in comparison with patients with CHD - 4.65 + 0.91 and 3.79 + 0.80 respectively (p <0.05).

Investigation of the level of expression of the mRNA gene of I κ B α in mononuclear cells reflects the level of transcriptional activity of NF-kB, and, accordingly, the severity of systemic inflammation of low intensity, in patients with stable coronary artery disease and CHD in combination with NAFLD showed a significant increase

(p <0.001) expression of the mRNA gene of I κ B α by 88.5% compared to patients with stable stable coronary heart disease (tab.2)

Table 2.
Expression of I κ Kappa B-alpha mRNA level

Group /Indicator, $X \pm \sigma$	Patients with CHD, n=25	Patients with CHD in combination with NAFLD, n=110
Експресія mRNA I κ B α , 2 ^{3ct}	0.0247 \pm 0.0165	0.215* \pm 0.048

Note: * - the probable difference as compared with data in patients with CHD (p <0.001).

The patients who have stable CHD and CHD in combination with NAFLD revealed increasing number of CEM CD32 + CD40 + in the blood stream (p <0.05) in comparing with group of almost healthy patients. This indicator had no significant changes in comparison with patients with CHD and patients with comorbid pathology (Table 3).

Table 3.
The content of circulating endothelial microparticles CD32⁺ CD40⁺ in patients with stable CHD and CHD in association with NAFLD

Group / Indicator, $X \pm \sigma$	Healthy patients, n=30	Patients with CHD, n=25	Patients with CHD in combination with NAFLD, n=110
CD32 ⁺ CD40 ⁺ , x 10 ⁷ /l	1.43 \pm 0.19	2.93 \pm 0.69*	2,95 \pm 1.94*

Note: * - the probable difference as compared with the data of apparently healthy persons (p <0,05).

Consequently, the analysis of functional state of the endothelium with help of CDM⁺ CD40⁺ has shown the presence of endothelial dysfunction in the groups of patients with CHD and CHD in combination with NAFLD.

Conclusions

Thus, comparison of indicators in systemic inflammation of low intensity and the marker of endothelial dysfunction in patients with CHD and CHD in combination with NAFLD revealed a significant increase in the level of TNF α , acute phase reactant and coagulation factor-FG and expression of the mRNA gene of I κ B α in patients with comorbid pathology was found testify to an increase in the level of systemic inflammation of low intensity in patients with coronary heart disease under conditions of NAFLD as compared to the group of patients with coronary heart disease.

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