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PREDICTORS OF MYOCARDIAL ISCHEMIA AND CENTRAL HEMODYNAMIC DISORDERS IN PATIENTS WITH CORONARY HEART DISEASE

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Coronary heart disease (CHD) ranks first among mortality causes in the world over the years [1]. Present scientific studies prove the leading role of chronic systemic inflammation (CSI) in the onset and progression of atherosclerosis (ASVD), which has been the morphological basis of CHD [2, 3]. Taking into account the above-mentioned, it is relevant to study the interaction of components of the pathogenetic process in the ASVD and CHD, the factors of formation and progression of the specified pathology in order to determine the diagnostic markers for the development and destabilization of these diseases and developing rational therapeutic approaches. The purpose of our research was to study the relationship between the indicators of systemic inflammation, the lipid spectrum of blood and the structural and functional condition of the heart in patients with stable CHD, and the search for predictors of its progression.

Materials and Methods. One-time, open-label, single group clinical trial involved 115 patients with CHD: stable angina pectoris, FC II, HF 0-I (62 men and 53 women aged 54±6.2). Patients were given laboratory and instrumental studies. The levels of total cholesterol, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), triglycerides (TG) by sedimentation, fibrinogen (Fg) concentration in blood by gravimetric analysis, the level of cytokines (CK) in the blood (interleukin 1β (IL-1β), tumor necrosis factor (TNFa), interleukin 10 (IL-10)) by the immune enzyme method, the content of circulating endothelial microparticles (EMP) of $CD32^+CD40^+$ in blood by cytofluorometry, mRNA gene expression of kappa B-alpha inhibitor (IkBa) of the nuclear kappa B transcription factor (NF-kB) in mononuclear cells of the peripheral blood by real-time polymerase chain reaction (Real-time PCR) were being determined [4, 5, 6]. Echocardiography and the 24 hour Holter ECG monitoring were performed by the standard method. A correlation and regression analysis was conducted.

Results. Moderate direct correlation between IL-1β level and blood lipid spectrum indicators, TNFa levels and LDL cholesterol levels has been detected. Fibrinogen (Fg) concentration was positively correlated with all investigated blood lipid spectrum indicators, as well as TNFa, IL-1β and IL-10 levels. Also, a direct moderate correlation between the amount of IL-1β and EMP $CD32^+CD40^+$, which characterizes inflammatory activation and endothelial dysfunction, was determined. The level of mRNA IkB expression positively correlated with the the level of cytokines, total cholesterol and LDL cholesterol in a moderate way. Investigation of the ratio of central hemodynamics indices and CSI markers revealed inverse correlation between left ventricular (LV) ejection fraction (EF) and TNFa ($r=-0.340$, $p<0.05$), LVEF and Fg ($r=-0.336$, $p<0.01$), direct correlation between LV isovolumetric relaxation time (IVRT) and EMP $CD32^+CD40^+$ ($r=0.311$, $p<0.05$), DT and EMP $CD32^+CD40^+$ ($r=0.383$, $p<0.01$), as well as inverse ones — between the ratio of the phases of the transmitral flow (E/A) and IL-1β ($r=0.333$, $p<0.05$). Analysis of the correlation between Holter ECG indicators and inflammatory markers revealed a direct moderate correlation between total ischemic burden (ΣtST depr), and TNFa and Fg, the same association was found in relation to the number of episodes of depression in the ST segment. To determine the independent predictors of cardiovascular risk regression analysis has been conducted. The values of TNFa and Fg were found to be prognostic markers affecting LVEF, proving the role of CSI in the development of LV systolic dysfunction: LVEF=60.74–0.33* TNFa–1.32*Fg. For the E/A parameter of the transmitral flow, the linear regression equation has had the form: E/A=0.96–0.02*IL-1β, for deceleration time (DT) — DT=199.17+3.72*EMP $CD32^+CD40^+$. The linear regression equation has also been obtained: IVRT=87.44+1.04*EMP $CD32^+CD40^+$. The indicated regression models prove the association of CSI markers and inflammatory endothelial activation with the development and progression of LV diastolic dysfunction. Myocardial ischemia prognosis has been presumable by CSI markers — TNFa and Fg: ΣtST depr =23.01+1.13* TNFa–4.58* Fg.

Conclusion. Thus, we have identified the central role of CSI in the pathogenesis of CHD, the relationship between CSI and dyslipidemia, endothelial dysfunction and coagulation potential of blood. According to the results of the correlation and regression analysis, it has been found out that CSI negatively affects the systolic function of LV and the components characterizing the diastolic function of LV (IVRT, DT, E/A), it has influence on the development of ischemic changes and electrical myocardial instability.

The data obtained are the basis for an active study of the efficiency of anti-inflammatory agents in patients with CHD in order to develop new pathogenetically based therapeutic approaches.

It is relevant to introduce actively into the clinical practice the use of certain predictors of myocardial ischemia and central hemodynamic disorders in order to stratify cardiovascular risk in patients with CHD.

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PREDICTION OF miRNA BINDING SITES IN GENES INVOLVED IN ALZHEIMER'S DISEASE

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miRNAs are small non-coding RNAs that regulate gene expression by interaction with target mRNAs. It was shown that miRNA binding sites are located not only in 3'UTR but also in 5'UTR and Coding Sequence (CDS) [1, 2]. There are plenty of programs for miRNA binding sites prediction in 3'UTR of mRNA [3, 4]. While miRNA binding sites in CDS and 5'UTR are not studied well. However, researching miRNAs and alteration of their concentration is an essential for understanding of miRNA involvement in the regulation of gene expression and pathological processes. It was shown that miRNAs are involved in Alzheimer's disease (AD) [5]. Among neurodegenerative diseases AD is the most common form of dementia in the world. World Alzheimer Report in 2015 estimates about 46.8 million people worldwide have dementia. Epidemiological data predict that by 2050 more than 131 million of people will be affected by AD. It was shown that some miRNAs were significantly differentially expressed between patients with AD and control groups [6]. Moreover, miRNAs can be used as potential biomarkers of AD [7]. Therefore, studying of miRNA binding sites in mRNA of genes involved in AD is essential for understanding molecular pathology of this disease.

Materials and Methods. Using databases and publications 74 most involved genes in Alzheimer's disease were chosen. Nucleotide sequences of these genes were taken from NCBI (http://www.ncbi.nlm.nih.gov). MirTarget program developed previously in our laboratory was used for miRNA binding sites prediction [8]. This program searches the miRNA binding site within entire sequence of mRNA in 3'UTR, CDS, 5'UTR taking into account the free energy of miRNA:mRNA hybridization (ΔG, kJ/mole). The ratio ΔG/ΔGm (%) was determined for each site (ΔGm equals to the free energy of miRNA binding with its fully complementary nucleotide sequence). Nucleotide sequences of 2568 miRNAs were taken from mirBase (http://mirbase.org) and 3701 miRNAs from article of Londin et al. [9]. miRNA binding sites with high ΔG/ΔGm ratio were chosen.

Results. It is predicted that among 74 genes involved in Alzheimer's disease development 64 are targets for miRNAs. Therefore, the expression of most genes associated with AD could be regulated by miRNAs. It was also revealed that binding sites of some of the miRNAs are arranged sequentially or with overlapping each other forming a cluster. Clusters were characterized according to their: 1) localization within mRNA; 2) average value of free energy of interaction; 3) compactness – a degree, which evaluate the ratio of length of all miRNA binding sites on the length of cluster in mRNA. There are eleven clusters with three miRNA binding sites in mRNAs of genes *ACHE* (in 5'UTR, CDS and 3'UTR), *APP* (in 5'UTR), *BACE* (in 5'UTR), *BIN1* (in 5'UTR), *CHAT* (in 5'UTR), *CYP46A1* (in CDS), *MAPT* (in 5'UTR), *MEF2C* (in 5'UTR), *MTHFR* (in 3'UTR). Four clusters with four miRNA binding sites were found in mRNAs of genes *CHRNB2* (in 3'UTR), *HMGB1* (in CDS and 3'UTR), and *SORL1* (in 5'UTR). Three clusters with five miRNA binding sites were found in genes *BACE1* (in 5'UTR), *CHRNA7* (in 5'UTR), and *PPARG* (in 5'UTR). There are two clusters with six miRNA binding sites in mRNAs of genes *TOMM40* (in CDS) and *PIN1* (in 5'UTR). Five miRNAs have binding sites in CDS of mRNA of *ACE* gene in position from 60 to 106 nucleotide (nt) with an average value of interaction energy equal to -126 kJ/mole. This cluster is 47 nt in length, 3.5 times less than the total length of all binding sites equal to 162 nt. There are four clusters of miRNA binding sites in mRNA of *ACHE* gene. Two of them are in 5'UTR of *ACHE* from 3 to 43nt and from 117 to 164 nt with an average ΔG equal to -118 kJ/mole and -122 kJ/mole respectively. Other clusters consist of binding sites for three miRNAs in CDS (from 1841 to 1869 nt with ΔG -124 kJ/mole) and in 3'UTR (from 2133 to 2162 nt with ΔG -119 kJ/mole). The mRNA of *GSK3B* gene contains three clusters of miRNA binding sites. First cluster of 41 binding sites for 23 miRNAs in 5'UTR from 2 to 40 nt with an average ΔG equal to -126 kJ/mole. Second cluster in 5'UTR from 352 to 378 nt with ΔG -123 kJ/mole. There is a cluster of polysites for 4 miRNAs in 3'UTR of *GSK3B* from 4705 to 4748 nt with ΔG -104 kJ/mole. The ratio of the total length of miRNA binding sites to the length of clusters in the mRNA of this gene is 22, 6 and 14 respectively. There are clusters in 5'UTR of following genes: *BIN1* has binding sites for miR-4-11761-3p, miR-8-21445-5p and miR-7-20193-5p; *PPARGC1A* has 23 binding sites for eight miRNAs with an average ΔG equal to -111 kJ/mole; five binding sites for three miRNAs in *PPARG* with ΔG -124 kJ/mole; 13 binding sites for nine miRNAs in *RELN* with ΔG -124 kJ/mole. Clusters of miRNA binding sites were found in 3'UTR of following genes: polysites for six miRNAs in *CD2AP* with average ΔG equal to -105 kJ/mole; binding sites for miR-101-27078-5p and miR-3-5147-5p in