Мікробіом кишечника при гострому коронарному синдромі

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Вступ. Кишковий мікробіом – відомий діагностичний показник і терапевтична ціль для неінфекційних захворювань. Мета цієї роботи – перевірити зв'язок між артеріальною ригідністю, еластичністю, кишковою мікробіотою або маркерами запалення в пацієнтів із гострим коронарним синдромом.

Методи. Еластичні властивості артерій оцінювали за допомогою неінвазивної артеріографії (Tensiomed, Угорщина), мікробіоту кишечника вивчали, визначаючи ключових представників методом рутинного культивування за допомогою наборів для біохімічної ідентифікації (LACHEMA, Чехія). Рівень SIgA та цитокінів визначали методом ELISA. Дані, отримані від клінічної експериментальної групи пацієнтів, порівнювали з результатами, отриманими від контрольної групи.

Результати. Отримані дані продемонстрували сильний взаємозв'язок між кишковою *E. faecalis* і PPbr (r=0,98), AIbr (r=-0,99) та AIao (r=-0,99). Достовірні кореляційні зв'язки виявлено для показників *E. coli* (lac+) та PPbr (r=-0,97), а також для *K. oxytoca* та CAT (r=-0,95), індексу систолічної площі об'ємної кривої (r=+0,99) та індексу діастолічної площі об'ємної кривої (r=-0,99). Індексовані ділянки об'ємної кривої корелювали з колонізацією *Streptococcus spp.* (r=-0,9 і 0,9 для систолічної та діастолічної площі відповідно). Артеріальний тиск співвідноситься з КУО *Candida spp.* (r=0,95).

Висновок. Виявлені бактерії (*E. faecalis, K. oxytoca*), прозапальні цитокіни та рівень SIgA в кишечнику, що корелює зі збільшенням артеріальної ригідності й еластичності, що спостерігається в пацієнтів із ГКС, можна використовувати як обґрунтування в профілактиці й індивідуальному лікуванні ГКС на ранніх стадіях захворювання.

Ключові слова: серцево-судинні захворювання, ішемічна хвороба серця, еластичні властивості кровоносних судин, мікробіом кишечника.

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Gut microbiome in acute coronary syndrome

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Introduction: The intestinal microbiome is a diagnostic indicator and therapeutic target for non-communicable diseases. The aim of this work is to test the relationship between arterial stiffness, elasticity, gut microbiota and inflammation markers in the patients with acute coronary syndrome.

Methods: Elastic properties of arteries were evaluated by non-invasive arteriography (Tensiomed, Hungary), and intestinal microbiota was studied with the determination of key representatives by routine culturing methodology using biochemical identification kits (LACHEMA, Czech Republic). The level of SIgA and cytokines were detected by enzyme-linked immunosorbent assay (ELISA). The data obtained for the clinical experimental group of patients were compared with the results obtained for the control group.

Results: Obtained data demonstrated a strong correlation between intestinal colonization by *Enterococcus faecalis* and PPbr (r=0.98), AIbr (r=-0.99) and AIao (r=-0.99). Significant correlations were found for *Escherichia coli* (lac+) and PPbr indicators (r=-0.97), and for *Klebsiella oxytoca* and SBP (r=-0.95), the systolic area index of the volumetric curve (r=+0.99), and the index of the diastolic area of the volumetric curve (r=-0.99). Indexed areas of the volumetric curve were correlated with colonization of *Streptococcus* spp. (r=-0.9 and 0.9 for systolic and diastolic area, respectively). Blood pressure correlated with the consentration of *Candida* spp. (r=0.95).

Conclusion: Detected bacteria (*E. faecalis, K. oxytoca*), pro-inflammatory cytokines, and intestine level of SIgA correlated with increased arterial stiffness and elasticity observed in patients with acute coronary syndromes (ACS) might be used as a rationale for the prevention and individual treatment of ACS in the earlier stages of the disease.

Keywords: Cardiovascular diseases, coronary heart disease, elastic properties of blood vessels, intestinal microbiome.

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Introduction

The progression of non-communicable diseases, such as type 2 diabetes, obesity, atherosclerosis, insulin resistance, etc., is associated with gut microbiome changes and their unconditional effects on the human body, including physiological and metabolic processes [1-3]. The relationship between intestinal microbiome changes and cardiovascular disease (CVD) pathogenesis has been considered [4]. Atherosclerotic CVD, which develops as a result of focal immunoinflammatory disease of large arteries caused by lipid accumulation, is the leading cause of mortality among adults both in Ukraine and worldwide. The progression of atherosclerotic lesions may occur either gradually and lead to the narrowing of arteries and hypoperfusion of organs and tissues or sharply – with the phenomena of atherothrombosis, acute occlusion of arteries and the development of critical ischemia leading to tissue necrosis [5-10]. In any clinical scenario of atherosclerosis development, immune reactions and chronic subclinical inflammation [11], which are modulated by numerous mechanisms, one of which is the interaction between the intestinal microbiome and the host's immune system, are important in supporting the pathological process [12,13]. Therefore, the intestinal microbiota is actively investigated as a diagnostic and therapeutic target for CVD [14]. However, the conducted studies mainly concerned patients with chronic forms of coronary heart disease (CHD). Data on the state of the intestinal microbiome in patients with acute coronary syndromes (ACS) are scarce in the literature [15-18], and similar studies have practically not been conducted in Ukraine.

Arterial stiffness and ventricular-vascular coupling are important physiological mechanisms for maintaining central hemodynamics and perfusion pressure in coronary arteries. The diagnostic and prognostic role of increased stiffness of large arteries has been well-studied in the general population and among patients with arterial hypertension. Still, there is a lack of studies considering the elastic-elastic properties of arteries in patients with CAD, including ACS [19].

The aim of the study was to investigate the relationship between the state of arterial stiffness and elastic properties of arteries and intestinal microbiota, as well as indicators of the immune response and inflammatory markers in patients with ACS.

Materials and Methods

13 patients participated in this descriptive study (7 men (53.8%) and 6 women (46.2%), median age 64 years, interquartile range [IQR] 55-71 years), who were hospitalized in the Zakarpattia Region Clinical Center of Cardiology and Cardiac Surgery of the Zakarpattia Regional Council. All patients underwent a standard general clinical examination (questioning and anamnesis, physical examination, resting electrocardiogram (ECG), echocardiography, and routine laboratory examinations). Coronary artery disease diagnosis was established per the Standards for providing medical care to patients with coronary artery disease [20] and was confirmed via angiography. All subjects received standard CHD treatment (antiplatelet agents, hypolipidemic agents, antianginal and antihypertensive medications). Arterial stiffness and elastic properties of the arteries were assessed in all patients using the non-invasive arteriography (Tensiomed, Hungary). The measurements were performed on patients placed in the supine position and after at least 10 minutes of rest. Blood pressure was measured on the dominant arm using the oscillometric method, and the recorded sphygmogram was used to calculate the following parameters of peripheral hemodynamics: brachial artery systolic and diastolic blood pressure (SBPbr and DBPbr, respectively), brachial artery pulse pressure (PPbr) and brachial artery augmentation index (AIbr). The following parameters of central hemodynamics were also recorded: systolic and pulse blood pressure in the aortic root (SBPao and PPao, respectively), augmentation index in the aorta (IAao), pulse wave velocity (PWV), and return time of the reflected wave to the aortic root (RT). The parameters of the volumetric pressure curve were also analyzed, particularly the indexed areas of the systolic and diastolic components of the volumetric curve. The study did not include patients with atrial fibrillation, frequent extrasystoles, and hypotension at the time of the examination (SBPbr less than 90 mmHg), nor people who took antibiotics at the time of the examination or within 30 days before the ex-

amination, patients with known inflammatory bowel disease or intestinal neoplastic processes, active gastrointestinal bleeding.

Intestinal microbiota was studied using serial dilutions of clinical samples on selective and chromogenic nutrient media. After cultivating at 37 °C for 24–48 hours, the bacteria colony-forming units (CFU) were counted. The biochemical identification of the pure cultures of isolated gut microbiota representatives was carried out using commercial Erba Lachema (Czech Republic) kits, namely ANAEROtest 23, ENTEROtest 24 N, NEFERMtest 24, CANDIDAtest 21, STAPHYtest 16, and STREPTOtest 24.

Circulating parameters of the immune response and markers of inflammation were measured in sera by solid-phase enzyme-linked immunosorbent assay (ELISA) method, and the absorbance was measured at 450 nm (BioTek Elx800, Biotek, USA)

The results obtained in the ACS patients group were compared with those obtained in control patients. The control participants were recruited using the case-control method to match participants by age and sex but without documented coronary artery disease.

Statistical analysis of obtained data was performed through descriptive statistics and statistical inference, using the Anaconda – Python environment and the OriginPro software package from OriginLab for numerical data analysis. Data correlation analysis and comparison methods of two or more dependent or independent data groups were performed using the non-parametric statistical models, namely the Mann-Whitney, Wilcoxon, and Kruskel-Wallis models. The results were considered statistically significant at the $p<0.05$ level.

Results

Most patients (76.9%) in the study sample had arterial hypertension, and one (7.7%) had type II diabetes. The median systolic and diastolic blood pressure were 140 (130-151.3) and 80 (77.5-90) mmHg, respectively. The mean body mass index was 31.4 kg/sq.m, and three patients (23.1%) had obesity. According to the coronary angiography data, single-vessel coronary artery disease was detected in 3 patients (23.08%), two-vessel disease in 4 patients (30.77%), and multi-vessel disease in 6 patients (46.15%). The analysis results of the arteries' elastic properties are presented in Table 1.

Patients' microbial were examined during the study (Fig. 1).

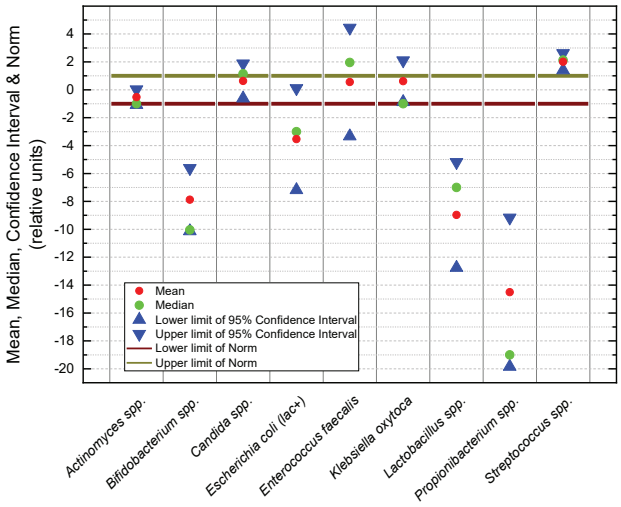

Figure 1. – Shifts of gut microbiota for ACS patients from reference ranges

Table 1

Indicators of elastic properties of arteries in ACS and control patients

Parameter	Patients with ACS (median, [IQR])	Control group
SPBbr, mmHg	125,0 (120,0; 136,0)	123,0 (117,0; 132,0)
PPbr, mmHg	44,0 (38,0; 50,0)	41,0 (37,0; 51,0)
AIbr, %	-8,8 (-22,7; -3,3)*	-12,9 (-30,5;-5,8)
SBPao, mmHg	123,0 (109,5; 137,5)*	113,0 (101,0; 125,5)
PPao, mmHg	39,9 (36,9; 54,8)*	30,1 (28,7; 46,8)
AIao, %	30,2 (27,7; 33,7)*	20,8 (12,7;29,3)
PWV, m/sec	12,0 (10,1; 12,1)*	9,3 (8,4;11,5)
RT, msec	95,0 (90,0; 105,0)	90,0 (84,0; 103,0)

Data are presented as median (interquartile range, IQR); * $p<0,05$.

The gut microbiota of patients with ACS is dominated by *Enterococcus* and *Streptococcus*, with a significant diversity of commensal and opportunistic microorganisms, namely *Enterobacteriaceae*, *Bifidobacterium*, *Lactobacillus*, *Propionibacterium* and *Candida*.

We observed an increase in *Streptococcus* levels (the actual average was 2 times higher than the allowable excess range of the normal average) while a decrease in *E. coli* (lac+) levels (the actual average was over 3.5 times below the standard average allowable decrease), *Propionibacterium spp.* (the actual average value is more than 14 times lower than the standard average allowable reduction), *Lactobacillus spp*. (the actual mean value was more than nine times lower than the allowable reduction value of the standard mean value) and *Bifidobacterium spp.* (the actual average is more than eight times lower than the allowable reduction in the standard average). It should be noted that the real average values of *Enterococcus faecalis, Klebsiella oxytoca* and *Candida spp*. were close to the permissible exceedance of the normal average.

Streptococcus (*Streptococcus parauberis*, *Streptococcus acidominimus*, *Streptococcus sanguis*, *Streptococcus anginosus*, *Streptococcus bovis*) was the most numerous coccal form of microorganisms. Bacteria of the genus *Actinomyces* were also found in normal limits in the microbiome of all ACS patients. In addition, bifidobacteria and lactic acid bacteria (*Lactobacillus catenaformis, Lactobacillus acidophilus*, and *Lactococcus lactis*) were isolated. However, their number was significantly lower than the normal values.

Correlation analysis revealed a strong correlation between intestinal colonization by *E. faecalis* and PPbr (r=0.98), AIbr (r=-0.99) and AIao (r=-0.99). Significant correlations were also found between the number of *E. coli* (lac+) and PPbr indicators (r=-0.97), as well as between the number of *K. oxytoca* and the indicators of SBP (r=-0.95), the systolic area index of the volumetric curve (r=+0.99), the index of the diastolic area of the volumetric curve (r=-0.99). In addition, indexed areas of the volumetric curve were correlated with the colonization of *Streptococcus spp. (*r=-0.9 and 0.9 for systolic and diastolic area, respectively). Mean BP was correlated with the number of *Candida spp*. (r=0.95). In the studied group of patients, we did not find correlations between indicators of intestinal microbial colonization and values of biomarkers of myocardial necrosis and echocardiography parameters.

In the comprehensive investigation of immune response parameters and inflammation markers, we observed that the levels of total IgA in the serum, as well as the concentrations of TNF-α and IL-12, remained consistently within the established reference ranges across all subjects included in our study.

Analyzing the parameters of secretory immunoglobulin A (SIgA) in coprofiltrate, a significant decrease was noted (5.33±1.2 mg/mL with a norm of 23.2–63.5 mg/mL). Instead, IL-1β and IL-10 levels were elevated. Thus, IL-1β concentration was 42.4±1.8 pg/mL for reference values of 0 - 3.9 pg/mL, and the level of IL-10 was 59.4±2.9 pg/mL for reference values of 3.9 -13 pg/mL.

Therefore, an increase in arterial stiffness is correlated with a decrease in the production of secretory immunoglobulin SIgA in the intestines and an increase in the production of IL-1β and IL-10. The data obtained coincides with the data from our previous studies [17].

Discussion

In recent years, detecting changes in the gut microbiome and its role in developing non-communicable diseases has become extremely important [21]. Our previous study investigated the gut microbiota of patients with non-communicable diseases associated with chronic inflammation (i.e., obesity, T2D, atherosclerosis, and cardiovascular disease) [22]. It was found that an increased number of enterococci and *Lactobacillus spp* and a decreased level of *E. coli* were characteristic features of the gut microbiota composition of patients with obesity. In the gut microbiota of patients with type 2 diabetes, a decrease in number of *E. coli* and lactobacilli was detected. Increased numbers of streptococci, enterococci and *Enterobacteriaceae* were observed in patients with arteriosclerosis. In contrast, increased numbers of staphylococci and *Candida spp.* and decreased numbers of *E. coli* were also observed in patients with cardiovas-

cular disease. A reduction in *Bifidobacterium spp., Bacteroides spp., Roseburia enterica* and *Akkermansia muciniphila* was observed in all studied groups of patients [22].

The results of this study allowed the investigation of associative relationships between the gut microbiota composition and the clinical and hemodynamic characteristics of patients with ACS. Specifically, in the study group, where most of the patients were overweight or obese, we found a strong positive correlation between *E. faecalis* colonization, shoulder circumference (r=0.89) and age (r=0.98), which may indicate a potential role of *E. faecalis* in cardiometabolic disorders.

The proposed mechanisms for the observed associations between the status and gut microbiota composition and parameters of arterial function and other characteristics of patients with ACS include the deleterious effects of microbiota metabolites and toxins on the endothelial wall and immune cells (e.g., trimethylamine, TMA and lipopolysaccharide, LPS) primarily [23] coupled with the lack of beneficial metabolites (e.g., short-chain fatty acids, SCFA) [24]. When absorbed in the gut, trimethylamine (TMA), the bacterial metabolite of dietary L-carnitine, choline, and phosphatidylcholine, undergoes oxidization in the liver and its oxidized form (TMAO), a potent promoter of atherogenesis in arteries and vascular wall remodeling [25]. A recent study confirmed that several microbiome strains, including *K. oxytoca* and 2 strains of *Enterococcus* genus, can metabolize choline and produce TMA due to the presence of the *cutC* gene, which encodes a glycyl radical enzyme that catalyzes the C–N bond cleavage [26].

A strong positive correlation between the number of *K. oxytoca* and the number of affected coronary arteries (r=0.93), and the correlations between greater colonization with *E. faecalis, K. oxytoca* and the increase in arterial stiffness, worsening of arterial elastic properties and reciprocal changes in the diastolic and systolic area of the pulse curve, observed in our study, supports the notion that TMAO is indeed the main mediator in the pathophysiological processes of ACS in our cohort. TMAO has been proposed previously, in other cohorts of ACS patients, as an independent risk factor and a prognostic marker for incident cardiovascular events [27]. The promotion of cytokines production in monocytes, macrophages and endothelial cells is one of several molecular mechanisms of the detrimental effects of TMAO [28], which may be, at least partly, the contributing factor in the reported inflammatory status of patients in our cohort.

Importantly, the observed correlation between the microbiota composition and clinical characteristics of ACS patients and the notion of the mechanisms and mediators involved provide a rationale for additional and complementary approaches in preventing and treating ACS and other cardiac artery diseases (CAD) [28]. Potential microbiome-relevant strategies are targeted either at the modulation of microbiota composition and promotion of SCFA production via probiotic and postbiotics administration [29, 30] or at the attenuation of TMA synthesis in the gut, by inhibiting the bacterial TMA-lyase enzyme, with either synthetic [31] or dietary agents [32], or its conversion to TMAO in the liver via inhibition of FOX3 enzyme activity [33]. Equality is important in the comprehensive understanding of the effects of the existing therapeutic agents on microbiota and potential interaction in the observed ACS outcomes.

Finally, the observed data further support the crucial role of diet in preventing CVD, mediated by the effect of dietary bioactives on gut microbiome.

In conclusions: An increased arterial stiffness observed in patients with ACS correlates with intestinal colonization by *E. faecalis*, *K. oxytoca*, a pro-inflammatory shift in serum cytokine profile, and a decrease in the production of secretory immunoglobulin SIgA in the intestines and rationalize novel approaches in the prevention and treatment of ACS and its consequences.

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