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EFFECT OF KALLISTATIN AND GHRELIN ON THE FORMATION OF ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH CHRONIC PANCREATITIS AND ATHEROSCLEROSIS

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ABSTRACT

Introduction: The article is devoted to the optimization of complex diagnosis of endothelial dysfunction in patients with a combination of chronic pancreatitis and atherosclerosis.

The aim: To study the level and effects of kallistatin and ghrelin on the formation of endothelial dysfunction in patients with chronic pancreatitis and atherosclerosis.

Materials and methods: 54 patients with chronic pancreatitis were examined. The serum kallistatin level was determined by immunoassay using the Human Serpin A4 ELISA Kit from RayBiotech according to the application method. The serum ghrelin level was determined by immunoassay using the Human/Mouse/Rat Ghrelin Enzyme Immunoassay Kit from RayBiotech. Endothelial dysfunction was determined by the method proposed by D.Celermajer.

Results: The study of endothelial-dependent and endothelial-independent vasodilatation is indicative of the presence of a pronounced ndothelial dysfunction in patients with chronic pancreatitis and atherosclerosis, which was manifested by a decrease in their level to 8.7±0.4% and 16.8±0.7%, respectively. The level of kallistatin and ghrelin in patients with chronic pancreatitis and atherosclerosis (15.44 ± 3.97 ng/ml and 276.69 ± 10.06 ng/ml respectively) also confirmed their important role in the formation of endothelial dysfunction in these patients.

Conclusions: The study of ghrelin and kallistatin level in serum can serve as a criterion for determining the severity of chronic pancreatitis and atherosclerosis, the development of endothelial dysfunction, and be a marker for predicting their future course.

KEY WORDS: Chronic pancreatitis, atherosclerosis, endothelial dysfunction, ghrelin, kallistatin

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INTRODUCTION

The endothelium is a multi-level cellular structure that permeates all organs and systems of the body. A significant influence on the endothelium has a state of lipid metabolism. Endothelial cells contain receptors for low-density lipoprotein (LDL), being a natural reservoir for binding their excess. Constantly interacting with peripheral blood cells, the endothelium controls the migration of cells to the depth of the vascular wall. Since blood flow depends on the diameter of the vascular bed, the balance of the coagulation and anticoagulation system factors produced by the endothelium is of fundamental importance [1].

The protective role of kallistatin in the vessels and organs is provided by vasodilation, inhibition of angiogenesis, inflammation, oxidative stress, apoptosis and fibrosis in the tissues of the body. Kallistatin is the key to inhibiting the use of kallikrein tissue and stimulating endothelial nitric oxide (NO) synthase (eNOS), sirtuin 1 (SIRT1), and signaling suppressor of 3 cytokines (SOCS3). Mechanically, kallistatin inhibits vascular inflammation through interaction with a transcription factor such as the Kruppel-like factor 4, which results in increased expression of eNOS and in NO levels in endothelial cells. At the same time, the exhaustion of endogenous kallistatin enhances organs` damage, enhances oxidative stress, inflammation, and fibrosis [2].

The expression and distribution of kallistatin in endothelial and smooth muscle cells of the blood vessels confirm its effect on the cardiovascular system. Increase in oxidative stress and reduction in the bioavailability of NO are important factors contributing to the pathogenesis of such systemic diseases as atherosclerosis, and also create conditions for the progression of the inflammatory process in the pathology of individual organs (including, in chronic pancreatitis) [3].

Ghrelin, as a multifunctional peptide hormone, that participates in the formation of eating behavior, energy balance, regulation of carbohydrate and lipid metabolism, as well as in modulation of the gastrointestinal tract functioning [4]. According to recent studies, ghrelin has potent anti-inflammatory properties by inhibiting proinflammatory cytokines such as interleukin-1, interleukin-6 and tumor necrosis factor, and binding of mononuclear cells to human endothelium [5].

THE AIM

The aim of the research – to study the level and effects of kallistatin and ghrelin on the formation of endothelial dysfunction in patients with chronic pancreatitis and atherosclerosis.

Table I. Doppler changes in BA in examined patients with pulmonary arterial hypertension and atherosclerosis, as well as control group

Indicator	Control group (n=30)	Patients on HP	
		I group (n=31)	II group (n=23)
	M ± m	M ± m	M ± m
Diameter of BA at the beginning of the study, mm	4,52±0,04	4,37±0,05	3,99±0,03*
Diameter for 30 seconds of reactive hyperemia, mm	6,42±0,05	5,31±0,03^	3,66±0,02*
Diameter for 60 seconds of reactive hyperemia, mm	6,23±0,08	4,61±0,05	3,88±0,03*
Speed of blood flow by BA, cm / sec	98,6±3,0	80,1±3,5	69,9±3,2*
EDVD (%)	13,8±1,05	10,5±0,7	8,7±0,4*
EIVD (%)	28,2±1,1	22,6±0,3	16,8±0,7*

Note: statistically significant difference between the indices in patients of the II group and the control group: * - p <0,05; statistically significant difference between the indicators in patients from groups I and II: ^ - p <0,05.

MATERIALS AND METHODS

54 patients with chronic pancreatitis underwent examination in the gastroenterological department of the Transcarpathia Regional Clinical Hospital named after A. Novak and in the family outpatient clinic by a gastroenterologist. The control group consisted of 30 practically healthy persons without signs of pancreatic-duodenal zone damage. The patients were from 30 to 61 years old. There were 39 (72.2%) male and 15 (27.8%) female among patients.

The diagnosis of chronic pancreatitis was based on complaints, anamnestic data, laboratory and instrumental methods of examination in accordance with the Marseilles-Roman`s criteria (1989), supplemented by Ya.S. Zimmermann (1995), as well as since the points system M-ANNHEIM.

The diagnosis of atherosclerosis was based on measuring of thickness of the intima-media layer of the carotid artery, the presence of the lipid profile violations and atherogenicity coefficient.

The serum`s kallistatin level (Serpin A4) was determined by immunoassay using the Human Serpin A4 ELISA Kit from RayBiotech, according to the methodology. An analysis of the results was carried out at a wavelength of 450 nm.

Serum ghrelin levels were determined using an enzyme-linked immunosorbent assay according to the implementation method. The human immunoassay kit RayBiotech Human / Mouse / Rat Ghrelin Enzyme Immunoassay Kit was used for the study. Evaluation was performed at a wavelength of 450 nm.

Endothelial dysfunction (ED) was determined according to the method proposed by D.Celermajer through detection of endothelial-dependent vasodilatation (EDVD) of the brachial artery (BA). EDVD was researched through the formation of reactive hyperemia by applying a cuff on distance from the site of the study. The diameter of the BA was measured after 10-15 minutes after rest. As a normal reaction, an increase in the diameter of BA on60-90 seconds against a background of reactive hyperemia was estimated at 10% or more. For the study of endothelial-independent vasodilatation (EIVD), the patient received 0.5 mg of nitroglycerin sublingually (as an endothelial-independent relaxation stimulus of peripheral vessels). Measurement was repeated in 2 and 5 minutes after taking nitroglycerin. EIVD was diagnosed when BA expansion in reactive hyperemia was significantly less than that of nitrates.

All patients were divided into 2 groups: group I included 31 patients with CP without atherosclerosis, group II - 23 patients with CP and atherosclerosis.

The methodology of studies corresponds to the Helsinki Declaration. The statistical processing of the patients` results was carried out using program STATISTICA 10.0 (firm StatSoft Inc., USA).

RESULTS

An ultrasound duplex scan of BA was performed in all patients and the EDVD and EIVD of BA were determined to detect ED. Table I shows the results of this survey.

According to results presented in Table I, the presence of ED was observed in all patients with CP and atherosclerosis. At the beginning of the study, there was a significant decrease in the diameter of the BA to 3.99±0.03 mm in patients with CP and atherosclerosis in comparison with the control group (4.52±0.04 mm) by duplex scanning of the BA. Patients with a CP without atherosclerosis also showed a tendency to decrease in diameter (4.37±0.05 mm), but these data were not statistically significant in comparison with the control group. Also, in patients with CP and atherosclerosis, there were more pronounced changes in the diameter of BA in 30 and 60 seconds of the study compared with the group of patients with pulmonary arterial hypertension without atherosclerosis and control group were observed. The EDVD study also indicates that there is a pronounced ED in patients with CP and atherosclerosis, which was manifested by a decrease in its level to 8.7±0.4% compared with 13.8±1.05% in the control group, respectively. Analyzing the indices of EIVD in patients with CP and atherosclerosis, the changes in comparison with the group of patients with CP (without atherosclerotic lesions) and control group (16,8±0,7% versus 22,6±0,3% in group I and 28.2±1.1% in the control group, respectively).

The serum kallistatin and ghrelin level were studied in all patients to detect the effects of these hormones on the course of these diseases.

Table 2. Comparison of the ghrelin, kallistatin and EDVD parameters in the examined patients

Indexes	Patients with CP without atherosclerosis		Patients with CP and atherosclerosis	
Ghrelin-Kallistatin	r=0,881	p=0,039	r=-0,743	p=0,044
EDVD-Kallistatin	r=0,674	p=0,035	r=-0,635	p=0,005
EDVD-Ghrelin	r=0,706	p=0,024	r=0,675	p=0,002

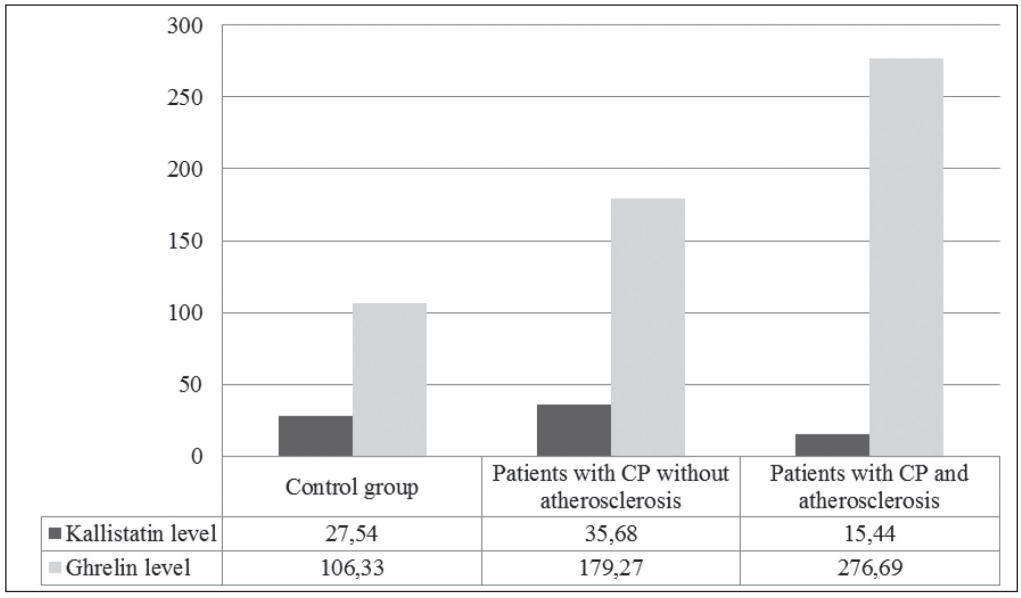

Fig. 1. Changes in the level of kallistatin and ghrelin in patients with CP and the control group

As can be seen from Figure 1, kallistatin level was increased in all patients with CP without atherosclerotic changes (up to 35.68±4.67 ng/ml) compared with the control group (27.54±3.95 ng/ml). Instead, a significant decrease in serum kallistatin (15.44±3.97 ng/ml) level in patients with CP and atherosclerosis, compared to the control group and the group of patients with CP without atherosclerosis were observed. An increase in serum ghrelin level to 179.27±9.84 ng/ml in patients with CP without atherosclerosis and to 276.69±10.06 ng/ml in patients with CP and atherosclerosis compared with the control group (106.33±9.43 ng/ml) were determined.

As shown in Table II, a positive correlation between the level of ghrelin and kallistatin (r = 0.888; p = 0.039), between the level of EDVD and the level of kallistatin (r = 0.674; p = 0.035), and between the level of EIVD and the level of ghrelin (r = 0.706; p = 0.024) were observe. A direct correlation between the level of EDVD and the level of ghrelin in patients with CP and atherosclerosis (r = 0.675; p = 0.002) and a negative correlation between the level of ghrelin and kallistatin (r = -0.743; p = 0.044) and between the level of EDVD and the level of kallistatin (r = -0.635; p = 0.05) were established.

DISCUSSION

In other author's studies of kallistatin was observed to inhibit inflammation in animal sepsis' models, myocardial ischemia-reperfusion, arthritis and salt-induced renal injury, etc. Kallistatin inhibits endothelial cell apoptosis and inflammation-induced organ damage by activating KLF4-eNOS 18, PI3K-AKT-eNOS and AKT-FOXO1 signaling pathways 19 and by preventing tumor necrosis factor-α (TNF-α)-mediated endothelial activation. It was shown that kallistatin inhibits atherosclerotic plaque formation caused by partial left carotid artery (PLCA) ligation in apoE-/- mice. Kallistatin also inhibits in plaques and the liver inflammation in vivo [6]. Other studies have shown kallistatin protective role in vascular aging. Kallistatin's anti-aging effect is mainly attributed to oxidative stress suppression by preventing miR-34a-mediated inhibition of antioxidant gene expression [7].

Due to its pleiotropic activity on energy metabolism, ghrelin has become a topic of great interest for experimental research. Furthermore, ghrelin seems to exert inhibitory effects on pancreatic acinar and endocrine secretory functions [8].

It is the first time when the level and effects of kallistatin and ghrelin on the formation of endothelial dysfunction in patients with CP were studied. Increase in the serum kallistatin level in all patients with CP without atherosclerotic changes confirms the hypothesis about the probable participation of kallistatin in the formation of organism`s protective mechanisms in the chronic inflammatory process in the pancreas, and its level can serve as a new biomarker for the diagnosis of CP. Significant reduction in serum kallistatin level in patients with CP and atherosclerosis provide the opportunity to assume the depletion of quinidine reserves in endothelial and smooth muscle cells of the body as a response to chronic systemic inflamma-

tory process with atherosclerosis and local inflammatory process in the pancreas in patients with CP.

Increase in the serum ghrelin level confirms its important role not only as an orexigenic hormone, but also its role in the pathogenesis of inflammation and oxidative stress in these patients.

Consequently, in response to inflammation (in the case of CP and atherosclerosis), protective mechanisms are triggered in the body, which are aimed in suppressing the inflammatory process. An increase in serum kallistatin levels, the key effect of which is inhibition of the kallikrein-kinin system, is likely to be considered as a response to inflammatory processes in the pancreas and vascular wall, which are aimed in limiting inflammation and preserving the integrity of the endothelial layer. However, with a combined and prolonged course of CP and atherosclerosis, there is an exhaustion of the compensatory possibilities of the organism and, accordingly, a decrease in its level in serum, which facilitates the process of chronicity and the formation of ED in such patients.

CONCLUSIONS

1. In patients with CP and atherosclerosis, an increase in serum ghrelin levels and a decrease in serum kallistatin level on the background of ED are observed with the results of EDVD and EIVD.
2. Patients with CP without atherosclerosis have an increase in serum ghrelin and kallistatin level.
3. The study of the ghrelin and kallistatin level in serum can serve as a criterion for determining the severity of CP and atherosclerosis, the development of ED, and be a marker for predicting their future course.

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