Article

Dysmicrocirculatory Phenomena in Patients with Acute Pancreatitis, Concomitant Diabetes Mellitus, in Association with Gene Polymorphism Enos (c774t)

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Abstract: Despite significant progress in the study of acute pancreatitis (AP), the components of its pathological mechanism are still in active search. Moreover, the risk of its progression and complications increases in the presence of metabolic syndrome, especially diabetes mellitus (DM). In patients with acute pancreatitis and diabetes mellitus, to establish the features of microcirculation disorders in association with polymorphism C774T of the eNOS gene. 56 patients with acute pancreatitis. Depending on the presence of diabetes mellitus, patients are divided into 2 groups: the first – without DM; and the second – with DM. The research methods included the determination of the severity of the disease on the APACHE–II scale, the state of the microcirculatory bed (LACK-02 (Lazma, Russia), and the genetic study of the eNOS gene (C774T) by real-time PCR (CFX96 Touch™ Real-Time PCR DetectionSystem, USA). Follow-up period: 1st, 3rd and 6th days of hospitalization at the clinic. The type of study is a prospective continuous sampling method. Acute pancreatitis in the early stages is accompanied by significant changes in microcirculation in the form of a decrease in tissue perfusion, depression of passive and active processes of regulation of vascular blood flow. These changes were associated with the presence of diabetes mellitus. In this condition, the nature of the disease was severe, and the changes in microcirculation were persistent and untreatable. It has been genetically established that microhemodynamic disorders in acute pancreatitis, with concomitant diabetes mellitus, are closely related to the C774T polymorphism of the eNOS gene. With T/T polymorphism, the degree of microcirculatory changes was recorded at the highest level relative to C/C and C/T and not the reverse. It is necessary to include a molecular examination of patients with AP, including those with diabetes mellitus, at an early stage to predict the risk of exacerbation of the disease and the development of complications.

Keywords: pancreatitis, diabetes mellitus, C774T, eNOS, microcirculation.

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1. Introduction

Acute pancreatitis (AP) is considered one of the most problematic nosologies in medical practice. The reasons for this are multifactorial pathogenesis, rapid involvement of other organs and systems in the pathological process, difficulty in diagnosis, ineffective treatment methods, especially with the development of complications [1].

In recent decades, diabetes mellitus (DM) has occupied a leading place among diseases of the endocrine system. Undoubtedly, the disease has both medical and socio-economic significance. Currently, there are great achievements in the establishment of pathogenesis, new methods of diagnosis and prediction of this pathology have been developed. However, advances in the treatment of diabetes mellitus still cannot fully meet modern requirements. The treatment of patients with complications is especially difficult [2, 3].

It is known that the main object of the implementation of negative pathogenetic actions in diabetes mellitus is microcirculation. Dysmicrocirculatory disorders in the form of angiospasm, stagnation of venous blood flow, formation of microthrombs and other complications accompany diabetes mellitus, which lead to ischemia of various tissue structures. This negative effect is most pronounced on the part of the lower extremities [4, 5].

Under physiological conditions, the formation of nitric oxide (NO) in the body is carried out in two modes. The basal (main) mode is involved in maintaining the tone of the smooth muscles

of the microcirculatory vessels and avoiding cellular adhesion to the vascular endothelium. Stimulated (induced) production is characterized by a short-term increase in nitric oxide secretion in certain conditions, in particular with cytokine activity, endogenous intoxication, tissue ischemia, etc. [6, 7].

One of the main enzymes of the production of the active secondary messenger NO, which plays a role in the regulation of vascular excitation, cell proliferation, leukocyte adhesion and platelet aggregation, is endothelial nitric oxide synthase (eNOS). This enzyme is encoded by the NOS3 gene, located on chromosome 7 in zone 7q35-7q36 [8].

The molecular substitution of cytosine for the nitrogenous base thymine in chromosome position 774 is known by the genetic marker C774T. As a result of this mutation, the secretion of eNOS increases, leading to a change in vascular tone, vasodilation of blood vessels, and an increase in the aggregation ability of platelets. These disorders lead to the formation of microthrombosis, the progression of the inflammatory process, and the development of complications [9, 10].

Until now, the study of the role of genetic disorders in the pathogenetic processes of acute pancreatitis has been little studied and is an urgent search in scientific papers.

Purpose. In patients with acute pancreatitis with concomitant diabetes mellitus, to establish the features of microcirculation disorders in association with polymorphism C774T of the ENOS gene.

2. Patients and Methods

A study of 56 patients with acute pancreatitis was performed on the basis of surgical departments of the State Medical Institution of the Katkov Republican Clinical Hospital (Saransk, Russia) and the Department of Faculty Surgery of the Ogarev Moscow State University (Saransk, Russia).

The type of study is prospective, conducted by the continuous sampling method.

The patients were divided into 2 groups depending on the presence of type II diabetes. The first group (clinical comparison) included 28 patients with AP. The second group (main, n=28) is similar to the first, but with DM.

The study also included 40 healthy people of both sexes (20 women (50.0%), 20 men (50.0%)) aged 18 to 50 years.

Criteria for participation in the study: age 27-65 years; gender – male and female; personal consent to the study; duration of the disease less than 72 hours; mild concomitant pathologies.

Exclusion criteria from the study: own refusal to participate; age younger than 27 or older than 65 years; duration of pathology more than 72 hours; severe concomitant diseases (oncological, psychological, etc.); surgical treatment.

It was clinically revealed that the average age of the subjects was 48.5±5.7 years (in the first group – 47.3±3.4 years, and in the second – 52.6±4.8 years).

By gender, it was revealed that there were men 33 (59,0 %) (16 (57,1 %) comparison groups and 18 (64.2%) - the main one), women – 23 (41.0%) (42.9% and 10 (35.8%), respectively).

The studied patients were prescribed drug therapy, including anti-enzyme, analgesic, detoxification, infusion, antibacterial, etc.

The research methods included:

determination of the severity of the studied patients using the APACHE –II scale. In patients of the comparison group, the final result of the APACHE –II scale was 3.54±0.14 points, and the main result was 11.5±0.23 points, which corresponds, according to the literature [11],to mild and severe severity, respectively;

assessment of the state of the microcirculatory bed, carried out using laser Doppler flowmetry at LAKK-02 ("Lazma", Russia). The test was performed in compliance with the standard recommendation of laser doppler flowmetry (LDF) [12]: room temperature - +22-24 °C; patient's position – calm, lying on his back; LDF measurement point – Zakharyin–Ged zone; LDF gram was recorded within 5-10 minutes. The following parameters were determined: coefficient of variation (Cv, ratio of tissue perfusion to its variability), microcirculation index (MI, value of tissue perfusion), microcirculation efficiency index (MEI, ratio of mechanisms of regulation of vascular blood flow);

The genetic study of the gene eNOS (C774T) was performed on real-time PCR (CFX96 Touch™ Real-Time PCR DetectionSystem, USA). The biomaterial of the test was venous blood. DNA samples were isolated using a set of reagents "DNA-Extran-1" (Syntol company, Moscow) using the Laura-Lee Boodram technique.

The study period: 1st, 3rd and 6th days of hospitalization at the clinic..

Statistical processing of the research results was performed using Microsoft Excel and Word 2013 and Statistica 12.0 digital programs. Data analysis was carried out according to the following criteria: kruskal–wallis (estimation of the average value of parameters), Student's (evaluation of quantitative indicators), Fisher's exact (determination of the significance of differences in qualitative indicators), Pearson coefficient (C') and odds ratio (OR) with a 95% confidence interval (95%

CI), the correlation coefficient is r. The hypotheses were considered statistically confirmed at a significance level of p<0.05.

3. Results and discussion

According to the results of the study, the early period of acute pancreatitis was accompanied by significant microcirculatory disorders. The degree of their changes had a strong association with the presence of diabetes mellitus (Table 1).

Table 1. Results of the microcirculatory test

Parameter	Norm (n = 40)	Study group	Study period, day		
			1st	3rd	6th
Cv, %	14,9±0,45	I (n=28)	11,1±0,57*	12,4±0,35*	15,01±0,72
		II (n=28)	8,2±0,19*1	10,4±0,27*1	12,4±0,38*1
MI, pf. units.	6,88±0,35	I (n=28)	4,7±0,19*	5,4±0,23*	6,3±0,44
		II (n=28)	2,2±0,11*1	3,8±0,15*1	5,2±0,22*1
MEI	1,97±0,19	I (n=28)	1,36±0,11*	1,55±0,14*	1,74 ±0,16
		II (n=28)	0,87±0,08*1	1,19±0,12*1	1,48±0,14*1

Note here and further: * – a significant difference to the norm group (p<0,05);1 – a significant difference to the first group (p<0,05)

All children with congenital pneumonia were in the emergency room on a ventilator. Saturation (Sat02) in newborns on the 1st day: Group 1-86.33%±2.04%, group 2-93.64%±0.6%. On objective examination, the gray color of the skin was in 83% of premature newborns and 56% of full-term newborns (p<0.05). Cyanosis of the skin was present in 48% of children of both groups, significantly more often in premature infants. Crepitating wheezes were heard in 37% of full-term children, in the study group only in 21% of children (p<0.05). In the general blood test, leukocytosis with an average value was more often observed in premature newborns-19,52*109/ l ±1.48, in full-term infants 22.37*109/l±2.03. The maximum values were reached in children with ENMT-85*109/ l, ONMT-49*109/l, whereas in 2 groups-44*109/l. But there were also children with leukopenia-7% of premature babies with average values of ENMT-4,3*109/ l±1.5, ONMT-1,3*109/ l±0.89 and 3% of full-term infants with an average value of 5*109/l±1.67 (Fig. 2). CRP is the main protein of the acute phase

It was found that the value of the coefficient of variation in patients of the comparison group was lowered relative to the reference level on the 1st and 3rd days of treatment by 47.2 and 24.8% (p<0.05). By the final days of observation, the Cv value was approaching normal.

In the second group, the level of the ratio of tissue perfusion to its variability was reduced at all stages of the study: on the first day - by 68.2% (p <0.05), on the 3rd day - by 52.7% (p <0.05). 05), and on the 6th day – by 31.1% (p<0.05).

The value of tissue perfusion (MI) in the early stages of the disease was reduced when compared with the norm by 25.4% (p<0.05) of patients in the first group and 44.9% (p<0.05) in the second group. In the subsequent stage (3rd day), the value of the microcirculatory indicator increased significantly in the comparison group, but it remained below the initial level by 18.1% (p <0.05). At the same time, in the main group the value of this indicator was not significantly increased; its value remained lower by 30.2% (p <0.05). By the end of the study, the PM value in patients of the 1st group approached the reference parameter, and the second group remained increased by 16.7% (p <0.05).

The ratio of mechanisms for regulating vascular blood flow in patients without diabetes seemed reduced in the first 3 days by 28.4 and 18.4% (p <0.05). On the final day it returned to the initial level (Table 1).

In the main group (AP + DM), the microcirculation efficiency index was significantly reduced by 54.2% on the first day (p<0.05). On the following day (3rd), an improvement in this indicator was observed by 37.3% (p<0.05), and on the 6th - by 22.1% (p<0.05) (Table 1).

The analysis of the genetic study showed that the results of alleles and genotypes of the polymorphism of the eNOS (C774T) gene in patients with AP and healthy people are presented in Table 2.

Table 2. Distribution of eNOS polymorphism (C774T)

Study group	Genotype, (n, %)			Allele, (n, %)	
	C/C	C/T	T/T	C	T

Norm (n= 40)	28 (70.0)	10 (25.0)	2 (5.0)	33 (82.5)	7 (17.5)
I (n=28)	14 (50.0)	13 (46.4)	1 (3.6)	20,5 (73.3)	7,5 (26.7)
II (n=28)	3 (10.7)	8 (28.5)	17 (60.8)	7 (25.0)	21 (75.0)
Total	17	21	18	27.5	28.5

It was found that the number of C and T alleles was normally 33 (82.5%) and 7 (17.5%), in the first group it was 20.5 (73.3%) and 7.5 (26.7%), in the second - 7 (25.0%) and 21 (75.0%). The frequency of genotypes C/C, C/T and T/T was 28 (70.0%), 10 (25.0%) and 2 (5.0%) in normal people, 14 (50.0%), 13 (46.4%) and 1 (3.6%) in patients in the comparison group, and 3 (10.7%), 8 (28.5%) and 17 (60.8%) in the main group (Table 2).

It should be emphasized that the mutant distribution of the T/T genotype of the eNOS (C774T) gene and its T allele in patients of the first group did not have a strong relationship with the norm group ($\chi 2$ =1.6, p=0.1). In the second group, a strong relationship (significant difference) was established between the T allele and the T/T polymorphism genotype relative to the reference group ($\chi 2$ =44.9, p=0.001).

Table 3. Multiplicative inheritance model (chi-square test, df = 1)

Indicator	Case	Control	$\chi 2$	p	OR	
	n=28	n=40			Mean	Mean
The first group						
Allele C	0.732	0.825	1.69	0.19	0.58	0.25 – 1.32
Allele T	0.268	0.175			1.72	0.76 – 3.94
Genotype C/C	0.500	0.700	1.71	0.19	0.43	0.16 – 1.17
Genotype C/T	0.464	0.250			2.60	0.93 – 7.29
Genotype T/T	0.036	0.050			0.70	0.06 – 8.16
The second group						
Allele C	0.250	0.825	44.97	0,001	0.07	0.03 – 0.16
Allele T	0.750	0.175			14.14	6.13 – 32.62
Genotype C/C	0.107	0.700	31.08	0,001	0.05	0.01 – 0.20
Genotype C/T	0.286	0.250			1.20	0.40 – 3.56
Genotype T/T	0.607	0.050			29.36	5.86 – 147.13

The authors claim that the polymorphic variant of the eNOS – T774T gene is considered to be the cause of increased NO secretion, the development of microcirculatory disorders, the aggravation of the general condition and the development of complications [13, 14].

According to the results of the genetic study, it was noted that patients with polymorphic genotype T774T of the eNOS gene were 18 (32,1 %, 1 (1,7 %) of the first group and 17 (30.3%) of the second).

The degree of microcirculatory changes was recorded at the highest level in patients with T/T polymorphism (MI – 1.78, Kv – 7.5, ISM – 0.82) when compared with the group of patients with C/C (4.9, 11.5, 1.39, respectively) and C/T (4.2, 10.3, 1.23, respectively) genotypes (Fig. 1).

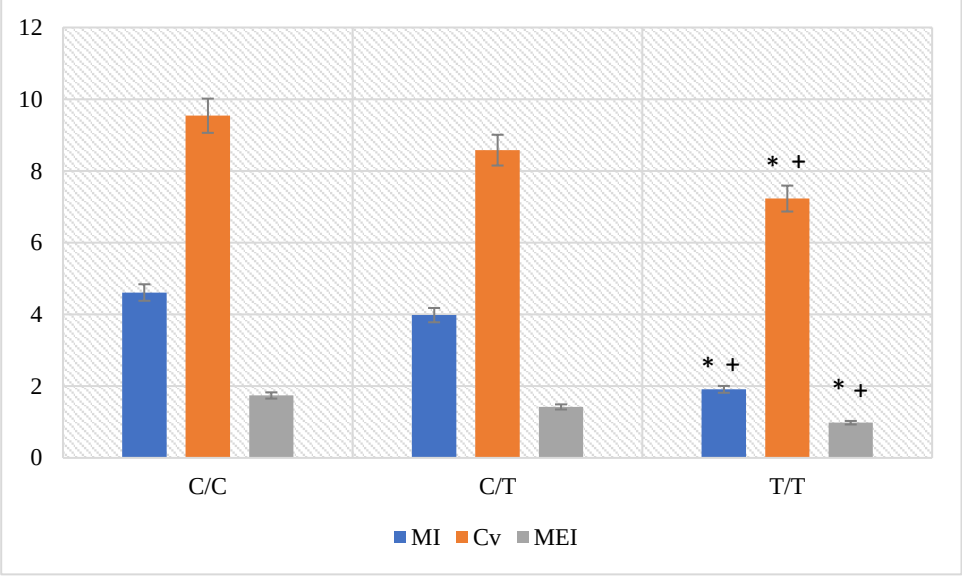

Figure 1. Microcirculation indices depending on the polymorphism of the eNOS gene

Analysis of the APACHE II scale showed that the final score of the T/T genotype subgroup was 14.5±0.88 (corresponding to a severe form). In the subgroups C/C and C/T genotypes, the APACHE II result was 2.8±0.11 and 4.1±0.24, which corresponds to mild severity.

The presence of diabetes mellitus with the mutant type T774T genotype of the eNOS gene had a more severe degree compared to carriers C774C and C774T.

So, the results of the study showed that the mutant genotype T/T of the eNOS gene was mainly detected in the second group (AP + DM), accounting for 60.7% (χ2=31.08, p=0.001). At the same time, a progressive decrease in vascular blood flow was recorded in this subgroup. Therefore, it is assumed that the T/T genotype of the eNOS gene may be a risk factor for worsening acute pancreatitis in the presence of diabetes.

The obtained material can undoubtedly be the basis for the use of personalized diagnosis and treatment of patients with acute pancreatitis concomitant with diabetes mellitus, using a molecular study of gene polymorphisms, especially those responsible for the functional activity of the microvasculature, which is given special importance in medical practice.

4. Conclusions

Acute pancreatitis in the early stages is accompanied by significant changes in microcirculation in the form of a decrease in tissue perfusion, depression of passive and active processes of regulation of vascular blood flow. Particularly pronounced dysmicrocirculatory phenomena occur with comorbid pathology – diabetes mellitus. In this condition, the nature of the disease was severe, and changes in microcirculation were persistent and untreatable.

It has been genetically established that microhemodynamic disturbances in acute pancreatitis accompanying diabetes mellitus are closely related to the C774T polymorphism of the eNOS gene. With T/T polymorphism, the degree of microcirculatory changes was recorded at the highest level relative to C/C and C/T and not in the opposite direction.

Application of artificial intelligence:

The article is written without the use of artificial intelligence technologies.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

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