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CHANGES IN THE PHYSICAL AND CHEMICAL PROPERTIES AND FATTY ACID COMPOSITIONS OF THE BLOOD SERUM IN PATIENTS WITH DIFFERENT COMMON PERITONITIS AS ONE OF THE CRITERIA FOR ASSESSING THE SEVERITY OF THE INFECTIOUS-INFLAMMATORY PROCESS

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The **aim** of this study was a clinical assessment of the diagnostic value of determining changes in the fatty acid composition and values of the surface tension of blood serum in patients with peritonitis in the light of assessing the severity of the course of the infectious and inflammatory process. In the course of the study it was found that the most informative indicator of the severity of the course of an infectious-inflammatory process is a sharp and prolonged decrease in the blood serum of patients with CCP in the level of γ-linolenic, dihomo-γ-linolenic fatty acids, as well as a persistent decrease in STC values. The results of the clinical study presented by us allow us to recommend, as a method of choice, to assess the severity of the course of peritonitis with the help of a comprehensive assessment of changes in the fatty acid composition and STC values of blood serum.

Keywords: peritonitis, fatty acids, surface tension coefficient.

Introduction. Despite the long history of studying peritonitis, various issues of its treatment remain one of the most difficult and not completely resolved problems of abdominal surgery. Given the large number of syndrome complexes developing in response to inflammation in the abdominal cavity, the approach to treatment is multidisciplinary in nature and includes a large set of measures aimed at both eliminating the source of peritonitis and correcting homeostasis disorders. The pathogenesis of peritonitis is a complex dynamic process of progression of pathophysiological disorders [8]. The main role in the pathogenesis of this severe complication is assigned to endogenous intoxication, caused by the accumulation in the body of toxins of microbial origin and metabolic products. It is the control of endotoxemia that often determines the outcome the disease.

In recent years, in the world of medical science, there has been an increasing interest in determining the physiological and biochemical status of a person when monitoring pathological processes developing in tissues, organs, and organ systems [1]. The physiological and biochemical status is determined by the presence at a certain stage and a certain situation of the concentration of biologically active compounds - proteins, fats and carbohydrates, as well as their metabolites [4]. In addition, many physiological and biochemical processes occur at the interface. A very important feature in the physical sense of the structural organization of living systems is a wide variety of dynamically stable and unstable interfaces [12]. These primarily include various

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membrane formations (cell membrane, lysosomes, mitochondrial, nuclear, and others), as well as the surfaces of blood vessels, visceral and parietal peritoneum, pleura, alveoli, blood cells [10].

As is known, the basis of endogenous intoxication accompanying peritonitis is the process of appearance in the bloodstream of substances with a pronounced detergent effect [3,9]. These substances primarily include, in particular, fatty acids (FA). It is known that fatty acids, especially polyunsaturated fatty acids (PFA), are involved in lipid peroxidation as substrates [5]. Due to the fact that in critical conditions, which include peritonitis, massive lipolysis occurs with an increase in the content of free fatty acids (FFA) in the blood serum, this leads to destabilization of cell membranes [6]. In turn, surface phenomena at the phase boundary also change, one of the physical indicators of which is the coefficient of surface tension (CST). However, data on the role of detergents and changes in the course of the infectious-inflammatory process of surface phenomena in the blood of patients with peritonitis are contradictory, which currently requires additional research and generalization of the data obtained.

The **aim** of the study. Clinical assessment of the diagnostic value of determining changes in the fatty acid composition and values of the surface tension of blood serum in patients with peritonitis in the light of assessing the severity of the infectious and inflammatory process.

Material and methods. The presented material is based on a clinical analysis of the results of treatment of 50 patients with common purulent peritonitis (CPP) who were treated in surgical hospitals of the Republican Hospital № 2 – Center for Emergency Medical Care (CEMP) of the Republic of Sakha (Yakutia) in the period from 2020 to 2023. The diagnosis of CPP was established on the basis of a standard clinical examination. The mean age of the patients was 35,6±5.1 years; there were 29 (58,0%) men and 21 (42,0%) women. To classify sepsis, in this case abdominal sepsis (AS), the criteria proposed by the conciliation conference of the American College of Pulmonologists and the Society for Critical Medicine Specialists ACCP/SCCM [1] were used. The choice of the spectrum for the determination of fatty acids was based on their prevalence and frequency of occurrence during destabilization of animal cell membranes. Hydrolysis and methylation of the presented fatty acids was carried out by gas-liquid chromatography [7]. Acid hydrolysis by Kenichi Ichihara and Yumeto Fukubyashi was used to obtain FA methyl esters [11]. For this purpose, 100 ml of blood serum were placed in sealed containers, 1 ml of 2,5% methanolic solution of H2SO4 was added and placed in a thermoshaker at 80°C and 1000 rpm for one hour. After cooling to room temperature (20°C), 1 ml of 0,9% NaCl was added to the resulting solution. Next, FA methyl esters were extracted with 0,5 ml of hexane. The resulting mixture was placed in a shaker for 1 min, then centrifuged for 1 min at 6,5 g. Methyl esters of FA were collected by decantation from the supernatant. 200 µl were taken for analysis. The hexane extract of FA esters was placed into the autosampler of a MAESTRO 7820/5975 chromatograph built on the basis of an Agilent 7820 gas chromatograph (USA) and a 5975 mass spectrometric detector from the same manufacturer. An HP-INNOWax capillary column was used for separation. Identification of FA methyl esters was carried out using a set of standards from Sapelco. 37-Component FAME mix (cat. no. 18919-1MP) and using the NIST database. Data collection was carried out using the Agilent ChemStation software. The concentration of methyl esters of fatty acids was determined from the area of chromatographic peaks of the corresponding compounds by the method of internal normalization. Data processing software used: Xcalibur (Thermo); spectral libraries: Mainlib; Microsoft Excel 2010. The static Du-Nouy method (on a Lauda TD1 tensiometer) was used to determine serum CST [2]. The inclusion criteria were: the presence of CCP, the presence of AS, the immediate causes of CCP were inflammatory and destructive diseases of the abdominal organs, the absence of a lethal outcome during the first 72 hours after the primary operation, the initial severity of the condition according to the Mannheim peritoneal index II-III degree. The exclusion criteria were: acute destructive pancreatitis with the development of peritonitis, neoplastic processes in the abdominal cavity, mesenteric thrombosis, initial severity of the condition according to the Mannheim peritoneal index less than grade II, death from peritonitis during the first 72 hours after surgery, the presence of fistulas.

Statistical processing of the material was carried out using the SPSS.Statistica.v22 software package. To determine the hypothesis and determine the type of distribution of the values of the studied features, the Shapiro-Wilkins test was used. In the groups to be compared, the mean values (X), standard deviation (s), confidence intervals and their fluctuations were determined. To study the relationship of quantitative traits, the Spearman correlation analysis method was used, since one of the variables, CST, did not obey the normal distribution law. The critical level of significance (p) when testing statistical hypotheses was taken equal to 0,05.

Results and discussion. Based on the results of the analysis of the FA profile and the assessment of the CST value of blood serum in patients operated on for CCP, it was found that in patients with severe CCP, the development of abdominal sepsis, the content of monounsaturated fatty acids (MFA) and PFA decreased sharply. At the same time, the level of unsaturated fatty acids (UFA) exceeded the control figures and averaged ΣUFA (77,22±1,1%). The high level of UFA was mainly due to the predominance of stearic [C 18:0] (49,19±0,5%), palmitic [C 16:0] (25,10±1,4%), myristic [C 14:0] (2,1±0,1%) and lauric [C 12:0] (0,83±0,2%) fatty acids, respectively. A parallel study of changes in the physicochemical properties of blood serum showed that with an increase in the severity of the condition of patients, there was a decrease in serum CST. A positive correlation was noted between the level of CST values and the severity of the condition (rs = +0,75), respectively.

The assessment of FA levels and CST values in patients with various types of abdominal sepsis showed that the nature of changes in the FA profile and the physicochemical properties of blood serum directly depended on the severity of the disease and developing complications. Thus, in the first 48 hours after surgery in patients with heavy sepsis (HS) and in the first 72 hours in patients with septic shock (SS) and multiple organ failure (MOF), the level of UFA in the blood serum exceeded the control figures by several times (especially significantly in patients with SS and MOF) and was in the blood serum of patients with HS - ΣUFA (78,17±1,4%), in the blood serum of patients with MOF – ΣUFA (81,15±1,6%). The increase in the level of UFA was mainly due to: stearic [C 18:0], margarine [C 17:0], palmitic [C 16:0], myristic [C 14:0] and lauric [C 12:0]. Along with an increase in the level of UFA, one could note consistently low numbers of CST in the blood serum. Thus, in the first 48 hours after surgery in patients with HS and in the first 72 hours in patients with SS and MOF, the level of CST values averaged: in patients with HS 41,2±1,1 mN/m, in patients with SS and MOF – 38,1±0,8 and 35,4±0,7 mN/m, respectively. The

dynamics of the content of fatty acids and the level of CST are presented in table.

When monitoring the level of UFA in the postoperative period, it should be noted that it largely depended on the effectiveness of complex therapeutic measures. So, with a favorable course of the postoperative period (the absence of a sluggish process, complications from the surgical wound or abdominal cavity), a gradual decrease in the level of UFA in the blood serum was observed and, on the contrary, an increase in the level of MFA and PFA. In cases where the level of MFA and PFA remained low for a long time (more than 72 hours), this always indicated an unfavorable course of the infectious-inflammatory process. Often in this case, progression of peritonitis or the development of severe complications with organ decompensation was observed. Similarly, there were changes in the physicochemical properties of the blood serum of patients with CCP. With a favorable course of the disease, the CST values of blood serum gradually approached the control figures. In cases of a complicated course, after some fluctuations in values, there was a progressive trend towards a decrease in serum CST. When considering the concentrations of some PFA, it was found that the level of ω3-PFA, such as cis-5,8,11,14,17-eicosapentaenoic [C 20:5Δ5,8,11,14,17] and cis-11.14,17-eicosatrienoic [C 22:3Δ11,14,17] decreased faster and more significantly than others in the case of an unfavorable course of the disease. Their concentrations were practically "trace" (0,0002±0,1% and 0,007±0,2), respectively. At the same time, it was possible to note an increase in ω6-PFA, mainly due to arachidonic [C 20:4Δ5,8,11,14] in comparison with other ω6-PFA and control values in patients with purulent peritonitis.

Thus, the total level of ω6-PFA was increased in more severe CCP (SS and MOF). A significant decrease in the coefficient ω3-PFA / ω6-PFA, mainly due to cis-5,8,11,14,17-eicosapentaenoic [C 20:5Δ5,8,11,14,17] and cis-11.14,17-eicosatrienoic [C 22:3Δ11,14,17] FA was observed during the entire period when AS events were present. Thus, the ratio of the coefficient ω3-PFA/ω6-PFA significantly decreased in patients with severe AS. More than 3 times in HS ($p<0,05$), more than 7 times in SS and MOF ($p<0,05$). Our data indicate that in patients with a more severe course of the disease, there are more pronounced disorders of the fatty acid composition of blood serum, mainly due to ω3 and ω6. At the same time, there is an increase in the UFA / MFA ratio, which is most pronounced during the first 72 hours after the operation. Such changes seem to be associated with the mobilization of unsaturated fatty acids, which are the first to be oxidized. Summing up the analysis of the FA profile in CCP, I would like to dwell on some features in the behavior of PFA at individual stages of treatment. When analyzing the concentrations of ω6-PFA, we encountered an unusual behavior of some of them. So, at admission and in the first 72 hours after surgery, the level of γ-linolenic [C 18:3Δ6,9,12], dihomo-γ-linolenic [C 20:3Δ8,11,14] FA in patients with HS and SS was extremely low, and in MOF they were present in the form of "trace" concentrations. In the case of a favorable course of the disease on days 7-10 from the moment of surgery, the level of γ-linolenic [C 18:3Δ6,9,12], dihomo-γ-linolenic [C 20:3Δ8,11,14] FA increased and already averaged 0,5 μg/ml and 1,7 μg/ml, respectively. This pattern was not observed in the behavior of other FA, in particular, UFA, MFA, and PFA. In our opinion, these changes are apparently associated with the features of the biosynthesis of unsaturated fatty acids. In addition, from literary sources [6], we know that γ-linolenic [C 18:3Δ6,9,12], dihomo-γ-linolenic [C 20:3Δ8,11,14] fatty acids in the human body are formed from linoleic [C 18:2Δ9,12] acid, which belongs to ω6-PFA. This transformation process requires the enzyme delta-6-desaturase (D-6-D), often the activity of which is suppressed by the excessive content in the blood of a large number of under-oxidized metabolic products, as well as the vital elements of microorganisms and their toxins. In addition, a frequent unfavorable sign of compensatory processes in CCP is a persistent increase in blood glucose levels and a decrease in blood insulin levels. There is evidence [9] that an excess of glucose in the blood blocks the activity of the D-6-D enzyme,

The content of fatty acids and the level of CST in the blood serum of patients common purulent peritonitis (% of the total fatty acids M±s)

Methyl ether FA and physico-chemical index	SIRS-3,4	HS	SS	MOF	Control
Linolenic, [C18:3Δ9,12,15]	0.21±0.03*	0.05±0.1*	0.04±0.1*	0.01±0.01*	0.27±0.02
cis-5,8,11,14,17-eicosapentaenoic, [C20:5Δ5,8,11,14,17]	0.002±0.1*	0.001±0.5*	0.0009±0.5*	0.0004±0.5*	0.032±0.5
cis-11-14-17-Eicosatrienoic, [C22:3Δ11,14,17]	0.01±0.5**	0.001±0.5**	0.009±0.01**	0.008±0.05**	0.03±0.1
γ-Linolenic, [C18:3Δ6,9,12]	0.001±0.07*	0.00096±0.15*	0.00089±0.15*	0.00037±0.07*	0.13±0.01
Linoleic, [C18:2Δ9,12]	10.33±0.01**	9.15±0.01**	7.05±0.01**	5.75±0.04**	16.11±0.05
Arachidon, [C20:4Δ5,8,11,14]	8.22±0.7**	10.33±0.3**	12.44±0.3**	15.66±0.5**	3.82±0.04
cis-8,11,14 -Eicosatrienoic, [C23:3Δ8,11,14] Dihomo-γ-linolenic	1.55±0.1**	2.00±0.1**	2.21±0.1**	3.59±0.6**	0.94±0.1
cis-13-16-Docosadiene, [C22:2Δ13,16]	0.0019±0.05*	0.0016±0.07*	0.0015±0.07*	0.0011±0.01*	0.02±0.03
cis-11-14-Eicosadiene [C20:2Δ11,14]	0.009±0.03*	0.013±0.03*	0.015±0.03*	0.019±0.01*	0.006±0.02
Σω3-PFA	0.22±1.1*	0.04±0.1*	0.03±0.1*	0.01±0.9*	0.62±0.01
Σω6-PFA	21.11±0.02*	22.71±0.05*	24.70±0.05*	25.02±0.01*	21.02±0.02
Σω3-PFA/Σω6-PFA, units	0.01±0.03*	0.003±0.01*	0.001±0.01*	0.0004±0.06*	0.03±0.01
Σ UFA	75.56±1.8*	75.17±1.4*	73.17±1.4*	72.94±1.1*	76.10±1.0
Σ MFA	1.11±0.03*	0.08±0.04*	0.05±0.04**	0.03±0.01*	2.26±0.01
CST (mN/m)	43±1.8*	41.2±1.1*	38.1±0.8*	35.4±0.7*	46.0±0.9

Note. * - the indicator significantly differs from the control ($p<0.05$), ** - the indicator significantly differs from the control ($p<0.01$).

followed by a critical decrease in the level of both γ-linolenic [C 18:3Δ6,9,12] and dihomo-γ-linolenic [C 20:3Δ8 ,11,14] FA. It should also be taken into account that γ-linolenic [C 18:3Δ6,9,12] and dihomo-γ-linolenic [C 20:3Δ8,11,14] fatty acids are involved in the synthesis of eicosanoids (prostaglandins) [10]. Prostaglandins are localized in almost all tissues and organs and are lipid mediators. Prostaglandins are synthesized from UFA and have a diverse effect, often directly opposite. In the course of the conversion of linoleic acid [C 18:2Δ9,12] into arachidonic acid [C 20:4Δ5,8,11,14], there are two steps in the cascade of prostaglandin formation. The first, in this case the key one, is carried out with the help of the enzyme D-6-D. The second, with the help of the enzyme delta-5-desaturase (D-5-D). With an increase in the blood level of underoxidized metabolic products, as well as microbial toxins, the D-6-D enzyme is inhibited, as a result, the synthesis in the cascade of γ-linolenic → dihomo-γ-linolenic UFA → anti-inflammatory prostaglandins (PG1) is disrupted. At the same time, the D-5-D enzyme is activated, which leads to the formation of pro-inflammatory prostaglandins (PG2).

Conclusions. Our observations showed that: 1. The total level of fatty acids in CCP was increased by 5-7 times relative to the control ($p<0,05$). At the same time, the coefficient of the ratio Σω3-PFA/ω6-PFA was significantly reduced in patients with HS by 2 times ($p<0,05$), and in patients with SS and MOF by more than 7 times ($p<0,05$). 2. The results of the study clearly show that in patients with a complicated course of the disease, there are more pronounced changes in the fatty acid composition of the blood serum due to ω3 and ω6 fatty acids, which persist throughout the entire period of existence of signs of abdominal sepsis. At the same time, the ratio of UFA/MFA increases with the severity of the course of the disease. Apparently, this is due to the fact that MFA are the first to be oxidized during lipolysis. 3. The most informative indicator of the severity of the infectious-inflammatory process is a sharp and prolonged decrease in the blood serum of patients with CCP in the level of γ-linolenic [C 18:3Δ6,9,12], dihomo-γ-linolenic [C 20:3Δ8,11,14] FA, as well as a persistent decrease in the values of the surface tension coefficient.

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