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PECULIARITIES OF IMMUNOLOGICAL AND METABOLIC DISORDERS IN CASE OF DIFFUSE PERITONITIS WITH DIFFERENT VARIANTS OF IL1 β (-511 C/T) GENE

Abstract. *The changes of immunological reactivity, functional activity of proteolytic, fibrinolytic, pro- and antioxidant systems in the development of widespread peritonitis depending on the genotype IL1 β (-511 C/T) encoding the synthesis IL1 β are investigated.*

Key words: *acute peritonitis, genotype, cytokines, IL1 β , fibrinolysis, proteolysis, lipid peroxidation, antioxidant protection*

Introduction. Acute peritonitis is one of the most topical issues in surgery. In spite of numerous studies of its causes, mechanisms of development, ways of progressing, many aspects of pathogenesis of an inflammatory process in the abdominal cavity are not investigated sufficiently making the choice of therapeutic tactics complicated and decreasing its efficacy [3, 6, 10]. In the first turn it is stipulated by the character of disorders of non-specific resistance, immunological reactivity, their correlation with marked morphological changes in the peritoneum, processes of formation and generalization of toxic metabolites resulting in considerable homeostasis disorders [2, 6, 7]. The correction of these processes would enable to make a positive influence on the course of general process in the peritoneum and improve the results of treatment of such patients.

In pathogenesis of peritonitis an important role is played by the mediators of inflammation – cytokines. There is a balance between pro- and anti-inflammatory mediators, and disorders of this equilibrium create preconditions for the development, spread and progress of the inflammatory process.

Recent studies have demonstrated that certain allele associations of genes of IL-1 family are responsible for the changed character of expression and production of appropriate proteins. IL1 β gene allele carrying point alteration in the promoter area in the position (-511) is associated with an increased production of this cytokine and its effect on the character of

inflammatory reaction. Three determinant genes of IL1 β (-511C/T) are known: CC-, CT- and TT-variant [1,4,9,10]. However, their connection with the course of the inflammatory process is not practically investigated. The blood plasma is known to contain a complex set of proteolytic enzymes, which correlation makes the basis of hemocoagulation, fibrinolysis, kininogenesis, immune reactions, regulation of circulation [2, 5, 6, 8]. Excessive activation of proteolysis is an important pathogenic link in the development of inflammatory reactions and disorders of hemostasis processes [2, 8]. Fibrinolytic system (FS) factors as inductors, mediators and regulators play an important role at all the stages of inflammatory process development [2, 8]. In this respect investigation of these systems with the development of inflammatory process in the abdominal cavity depending on the functional polymorphism of cytokine genes is rather topical.

A necessity arises to study gene polymorphism coding proteins of IL1 β family, their effect on the character of development and expressiveness of inflammatory reactions in case of diffuse forms of peritonitis.

The therapeutic tactics in case of diffuse peritonitis in patients with various variants of genetic determination of interleukin activity should be obviously different, although the studies available are not sufficient.

Objective: to investigate the correlation between the variants of IL1 β (-511 C/T) IL1 β (-511 C/T) and changes of immunological reactivity, functional activity, proteolytic, fibrinolytic, pro-

and anti-oxidant systems in the development of diffuse peritonitis and the variant of IL1 β (-511 C/T) genotype coding IL1 β synthesis.

Materials and methods. A comprehensive examination of 37 patients admitted to the hospital with the signs of diffuse peritonitis was conducted. The diagnosis was confirmed surgically.

All the patients underwent examination of the variants of IL1 β -511C/T gene polymorphism. The material for molecular-genetic examination was DNA isolated from the lymphocytes of the peripheral venous blood of patients by means of the set of reagents «DNA-sorb-B». Polymerase chain reaction (PCR) was conducted using Taq-DNA-polymerase and specific primers. The alleles were discriminated by means of specific endonucleases of AVAI and AVAII restriction («Fermentas», Lithuania) in hydrolysis reaction. PCR restriction products were divided by means of electrophoresis in 2% agarous gel with tris borate buffer (TBB) concentrated with ethidium bromide for 30-45 minutes: “mutant” AVAII-resistance T-allele was divided into “wild” C-allele [1]. The fragments were visualized by means of transilluminator with the molecular mass marker available 100-1000 bp («SibEnzym», Russia).

The level of cytokines in the blood serum was evaluated by means of immunoenzyme method on the analyzer STAT-Fax Plus-303 (USA); test systems DIACLON were used (France), DRG (Germany).

Fibrinolytic activity of the blood plasma was detected by means of examination of enzymatic fibrinolytic activity (EFA), non-enzymatic fibrinolytic activity (NFA) and total fibrinolytic activity (TFA). The main protein fractions constituted proteolytic activity: azoalbumin, azocasein, azocol. Peroxide oxidation and antioxidant defense were detected by means of examining Malone aldehyde in the blood plasma and erythrocytes, reduced glutathione, glutathione -S-transferase, glutathione peroxidase. These values were estimated by means of the standard sets of reagents produced by “Simko Ltd” (Lviv) according to the methods elaborated by O.L. Kukharchuk (1996), B.M. Bodnar et al. (2000).

The patients were distributed into three groups depending on IL1 β -511C/T gene polymorphism.

I group of patients included 3 individuals with CC-variant of IL1 β -511C/T gene polymorphism. II group included 28 patients with CT-variant of IL1 β -511C/T gene polymorphism. III group included 6 patients with TT-variant of IL1 β -511C/T gene polymorphism.

The control group included 15 practically healthy volunteers.

The results obtained were statistically processed by means of Student and Fisher criteria and probability ratio.

Results and discussion. The analysis of IL1 β concentration in the blood found that in patients with diffuse peritonitis this index increased reliably ($209,29 \pm 5,47$ pg/ml against $94,92 \pm 2,04$ pg/ml in the control; $p < 0,01$). It is indicative of an important role of IL1 β in progressing of the general process. At the same time, a clear correlation is found between the concentration of IL1 β in the blood and IL1 β 511C/T genotype variant. The lowest concentration of IL1 β was found to be at CC-variant ($192,71 \pm 5,08$ pg/ml against $94,92 \pm 2,04$ pg/ml in the control; $p < 0,05$). It was reliably higher than that of the control value but it was considerably lower than that of the general value and similar values at CT- and TT-variants. The concentration of IL1 β was reliably higher in patients with CT-variant ($232,31 \pm 4,08$ pg/ml; $p < 0,05$). The highest IL1 β concentration was found in patients with TT-variant of IL1 β 511 C/T gene ($263,45 \pm 6,15$ pg/ml), being reliably higher the similar value in the control, general index and that of the patients with CC- and CT-variants (Table 1).

Considering the fact that among the patients with diffuse peritonitis (Figure) the patients with CT- and TT-variants prevail (75,6% and 16,3% respectively) we have developed the method to prognosticate the course of peritonitis by the detection of variants of IL1 β 511C/T genotype – with CT- and TT-variants unfavourable course is predicted with quick spread of inflammatory process in the abdominal cavity [9].

Considering the fact that interleukins are triggers of different mechanism of inflammation we have examined the correlation between their concentration in the blood plasma and expressiveness of peroxide oxidation processes, antioxidant defense, proteolysis, fibrinolysis, as well as peculiarities with different variants of IL1 β

Table 1

IL1β concentration with different variants of IL1β (-511C/T) gene in patients with diffuse forms of peritonitis

№	Index	Control	General index	1 group (CC- variant)	2 group (CT- variant)	3 group (TT- variant)
		1	2	3	4	5
1	IL1β (pg/ml)	94,92 ± 2,04	209,29 ± 5,47 p 1-2**	192,71 ± 5,08 p 1-3* p 2-3**	232,31 ± 4,08 p 1-4*** p 2-4** p 3-4**	263,45 ± 6,15 p 1-5*** p 2-5** p 3-5*** p 4-5*

Note: * - probability ratio p <0,05; ** - < 0,01; *** - < 0,001 (only statistically reliable differences are presented).

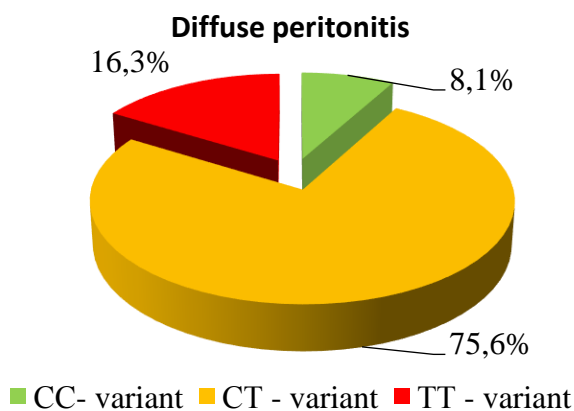


Figure. Frequency of detection of the variants of 1β - 511(C/T) interleukin gene polymorphism in case of diffuse peritonitis

511 C/T gene.

The investigations conducted are indicative of the fact that redox-system imbalance plays an important role in realization of damages in case of diffuse peritonitis, that is, correlation between pro- and antioxidant systems. The level of Malone aldehyde in the erythrocytes of the examined patients (Table 2) was found to be 22,37% higher (p<0,05), that was reliably higher than that of the control index (10,12 ± 0,25 mcM/L against 8,27 ± 0,34 mcM/L in the control; p<0,05). At the same time, the activity of the antioxidant system enzymes either increased inconsiderably (glutathione-S-transferase by 7,65%; p<0,05), or decreased considerably (activity of reduced glutathione – by 20%; p<0,05), which is indicative of imbalance in the redox-system.

We have found considerable differences of the redox-potential disorders in patients with different variants of IL1β (-511C/T) gene. The most pronounced increase of Malone aldehyde

concentration was found at CT- and TT-variants (25,88%) and (25,27%; p<0,05) respectively, while in patients with CC-variant this index was only 20,31% higher (p<0,05). Activity of reduced glutathione in patients with CT-variant was 46,55% lower (p<0,05), and in patients with TT-variant was 14,12% lower (p<0,05), while in patients with CC-variant this index did not practically differ from that of the control.

It is indicative of the fact that in case of diffuse peritonitis in patients with CT- and TT-variants the activity of peroxide oxidation processes becomes reliably higher against the ground of reduced activity of antioxidant defense enzymes, first of all reduced glutathione. This imbalance in the redox-system stipulates the necessity of its correction in the treatment of patients with diffuse peritonitis.

One of the mechanisms of damage in case of diffuse peritonitis is the system of unlimited proteolysis realizing its effect through excessive splitting of low molecular structures, distortion of proteolysis of middle molecular peptides including hormone regulatory substances, and excessive activation of collagen structures proteolysis. Thus, in case of diffuse peritonitis (Table 3) proteolytic activity of low molecular substrates increase according to the data obtained (up to 2,95 ± 0,25 E440/ml/hour against 2,47 ± 0,17 E440/ml/hour in the control; p<0,05). Although considerable differences of such increase are found in patients with different variants of IL1β (-511C/T) genotype. Thus in patients with CC-variant proteolytic activity by azoalbumin became 0,81% higher, with CC-variant– 6,88% higher (p<0,05), and with TT-variant 19,43% higher (p<0,01). It is excessive activation of proteolysis of low molecular structures that is

Table 2

Dynamics of indices of peroxide oxidation and antioxidant defense of the blood plasma in the groups of patients examined

№	Index	Control	General index	1 group (CC-variant)	2 group (CT-variant)	3 group (TT-variant)
		1	2	3	4	5
1	Malone dialdehyde in plasma (mcM/L)	3,48 ± 0,15	3,33 ± 0,16	3,55 ± 0,21	2,88 ± 0,27 p 1-4* p 2-4* p 3-4*	3,31 ± 0,42
2	Malone dialdehyde in erythrocytes (mcM/L)	8,27 ± 0,34	10,12 ± 0,25 p 1-2*	9,95 ± 0,33 p 1-3*	10,41 ± 0,41 p 1-4*	10,36 ± 1,04 p 1-5*
3	Reduced glutathione (mcM/ml)	0,85 ± 0,05	0,68 ± 0,03 p 1-2*	0,73 ± 0,02 p 1-3* p 2-3	0,58 ± 0,05 p 1-4** p 3-4*	0,73 ± 0,03 p 1-5* p 4-5*
4	Glutathione-S-transferase (nmol)	142,59 ± 4,83	154,42 ± 6,23	149,15 ± 8,16	166,36 ± 0,52 p 1-4* p 2-4* p 3-4*	150,75 ± 3,85
5	Glutathione peroxidase (nmol)	205,52 ± 7,23	301,03 ± 9,01 p 1-2**	321,26 ± 1,91 p 1-3** p 2-3*	274,11 ± 1,44 p 1-4* p 2-4* p 3-4*	315,6 ± 14,81 p 1-5** p 4-5*

Note: * - probability ratio $p < 0,05$; ** - $< 0,01$; *** - $< 0,001$ (only statistically reliable differences are presented).

Table 3

Dynamics of indices of the blood plasma proteolytic activity in the groups of patients examined

№	Index	Control	General index	1 group (CC-variant)	2 group (CT-variant)	3 group (TT-variant)
		1	2	3	4	5
1	Azoalbumin (E440/ml/hour)	2,47 ± 0,17	2,56 ± 0,09	2,49 ± 0,09	2,64 ± 0,21	2,95 ± 0,25 p 1-5* p 2-5* p 3-5**
2	Azocasein (E440/ml/hour)	1,21 ± 0,21	1,42 ± 0,08	1,38 ± 0,09	1,62 ± 0,14 p 1-4* p 3-4*	1,68 ± 0,14 p 1-5* p 2-5* p 3-5*
3	Azocol (E440/ml/hour)	0,46 ± 0,03	0,62 ± 0,05 p 1-2*	0,57 ± 0,06 p 1-3*	0,61 ± 0,06 p 1-4*	0,69 ± 0,09 p 1-5*

Note: * - probability ratio $p < 0,05$; ** - $< 0,01$; *** - $< 0,001$ (only statistically reliable differences are presented).

one of the causes of more pronounced signs of endotoxiosis.

Activity of proteolysis of middle molecular peptides in case of diffuse peritonitis increase as well (to $1,42 \pm 0,08$ E440/ml/hour against $1,21 \pm 0,21$ E440/ml/hour in the control; $p < 0,05$). Although in patients with CC-variant it increased only by 14,05% ($p < 0,05$), with CT-variant - 33,88% ($p < 0,05$), and TT-variant - 38,84% ($p < 0,05$). This excessive activation of proteolysis of middle molecular peptides which greater part plays a regulatory role of different mechanisms of inflammation, can be considered as a cause of discoordination between activators and inhibitors of damaging mechanisms, and it can be a cause promoting endotoxiosis.

While examining proteolytic activity to collagen structures we have found that in case of diffuse peritonitis this index increases by 25,81% (to $0,69 \pm 0,09$ E440/ml/hour against $0,46 \pm 0,03$ E440/ml/hour in the control; $p < 0,05$). At the same time, its biggest growth was found in patients with TT-variant (50%; $p < 0,05$), a little less with CT-variant - 32,61% ($p < 0,05$), and the least - with CC-variant - 23,91% ($p < 0,05$). It is activation of collagen structures proteolysis that is an important mechanism promoting the spread of inflammatory process in the abdominal cavity due to disorders of differentiation processes occurring with the participation of the connective tissue elements.

Activity of fibrinolytic system plays an important role in the processes of spread of inflammatory process in the abdominal cavity.

We have found (Table 4) that in case of diffuse peritonitis the total fibrinolytic activity increases by 76,03% (to $2,82 \pm 0,42$ E440/ml/hour against $1,46 \pm 0,07$ E440/ml/hour in the control; $p < 0,01$). At the same time, the most pronounced fibrinolytic activity was found in patients with TT-variant (93,15%; $p < 0,01$), less - with CT-variant (91,09%; $p < 0,01$) and the least - with CC-variant (63,01%; $p < 0,01$). It was fibrin splitting and destruction of fibrin junctions occurring in case of inflammation that did not enable to differentiate the focus of inflammation from other portions of the abdominal cavity promoting its spread. It is important to note that activation of fibrinolytic activity occurs mainly at the expense of non-enzymatic fibrinolysis that became 106,17% higher in the examined patients ($p < 0,01$), and the biggest growth of this index was found in patients with TT-variant (270,37%; $p < 0,001$), less in patients with CT-variant (212,35%; ($p < 0,01$) and the least - with CC-variant - 95,06% ($p < 0,01$). Although enzymatic fibrinolytic activity increased but it was not so marked (by 30,77%; $p < 0,01$). And in patients with TT-variant it was even lower than that of the control, which can be a sign of disorders of synthesis of these enzymes in the liver.

Therefore, the investigations conducted are indicative of the fact that the leading mechanisms of inflammatory process progressing in the abdominal cavity are excessive activity of IL1 β , carrying genetic determination. The examined cohort of patients with TT- and CT-variants dominates as IL1 β activity is the highest. It

Table 4

Dynamics of indices of fibrinolytic activity of the blood plasma in the groups of patients examined

№	Index	Control	General index	1 group (CC-variant)	2 group (CT-variant)	3 group (TT-variant)
		1	2	3	4	5
1	TFA (E440/ml/hour)	$1,46 \pm 0,071$	$2,57 \pm 0,13$ p 1-2**	$2,38 \pm 0,14$ p 1-3**	$2,79 \pm 0,25$ p 1-4**	$2,82 \pm 0,42$ p 1-5**
2	NFA (E440/ml/hour)	$0,81 \pm 0,031$	$1,67 \pm 0,12$ p 1-2**	$1,58 \pm 0,15$ p 1-3**	$1,72 \pm 0,19$ p 1-4** p 2-4**	$2,19 \pm 0,59$ p 1-5***
3	EFA (E440/ml/hour)	$0,65 \pm 0,051$	$0,85 \pm 0,06$ p 1-2*	$0,80 \pm 0,05$ p 1-3*	$1,07 \pm 0,12$ p 1-4** p 2-4* p 3-4*	$0,63 \pm 0,17$ p 4-5**

Note: * - probability ratio $p < 0,05$; ** - $< 0,01$; *** - $< 0,001$ (only statistically reliable differences are presented).

enables to suggest about genetic determination of inflammatory process in case of peritonitis. A clear dependence between the concentration of IL1 β (-511C/T) and activity of peroxide oxidation processes, antioxidant defense, proteolysis and fibrinolysis are indicative of close interrelations of these mechanisms in realization of inflammatory process in the abdominal cavity.

Conclusions 1. Intensity of inflammatory reactions and metabolic disorders in case of peritonitis is of genetically determined character and depends on the variants of IL1 β -511C/T gene. 2. The dependence found between the concentration of IL1 β (-511C/T) and activity of peroxide oxidation processes, antioxidant defense, proteolysis and fibrinolysis are indicative of close interrelations of these mechanisms in realization of inflammatory process in the abdominal cavity.

Prospects of further scientific search: to investigate other mechanisms of inflammatory reaction in patients with acute peritonitis depending on the variants of IL1 β (-511C/T) gene polymorphism.

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