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INFLAMMATION MARKERS AS RISK FACTORS OF EDEMATOUS PANCREATITIS DEVELOPMENT PROVIDING OF GENETIC DETERMINATION OF *IL-4* PRODUCTION

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Abstract

Introduction. The powerful mechanism of the immune system involvement in the pathogenesis of acute pancreatitis (AP) and exacerbation of chronic pancreatitis (ECP), especially from the positions of influence of polymorphism of genes which regulate the inflammatory response (interleukin -1β (IL-1β), -4 (IL-4), -6 (IL -6) and tumor necrosis factor-alpha (TNF-α), and others.) on the clinical course of pancreatitis still stays outside the attention of researchers.

Objective. The objective of our investigation was to study some indicators of systemic inflammatory response (TNF-α, IL-1β, CRP and α1-AT) in patients with AP and ECP depending on the gene interleukin 4 (IL-4, (C-590T).

Materials and methods. The study involved 101 patients with AP and ECP. Molecular genetic studies included the determining of polymorphic variants of gene *IL-4* (C-590T). The levels of interleukins IL-4, IL-1β and the tumor necrosis factor - alpha (TNF-α), CRP and α1-AT were determined.

Results. Past studies concerning the levels of IL-4 production caused by gene *IL-4* polymorphism, established the lower production of IL-4 in the *TT*-genotype owners of the gene *IL-4* (3.78 pg/ml vs. 31.91 and 19.31 pg/ml of *CC*- and *CT*-genotypes owners,

respectively; $p_{CC}<0.001$, $p_{CT}<0.01$). TNF-α concentration was higher in the carriers of the wild allele C-590T of gene *IL-4* polymorphism (11.25 and 10.54 pg/ml vs. 4.80 pg/ml of *TT*-genotype owners, respectively, Mann-Whitney criterion - 4.87, $p_{CC}<0.001$, $p_{CT}=0.002$). The high concentration of IL-1β in peripheral blood serum was found in 7.69% of *T*-allele carriers and 34.67% of the owners of *C*-allele of C-590T polymorphism of gene *IL-4* among AP patients ($p=0.008$). The frequency of patients with a higher concentration of *CRP* (>10 mg/ml) and α1-AT between allele carriers of gene *IL-4* (*T*- vs. *C*-allele) did not differ significantly: 61.54% vs. 70.67% ($p>0.05$) and 34.61% vs. 28.0% ($p>0.05$), respectively.

Conclusions. The increased production of IL-1β is a risk factor for AP in the *C*-allele carriers of *IL-4* gene and protective factor of AP development in *T*-allele carriers of *IL-4* gene (rs2243250) selected polymorphism.

Key words: gene, polymorphism, pancreatitis, IL-4, IL-1β, TNF-α, CRP, α1-AT.

Acute pancreatitis (AP), and exacerbation of chronic pancreatitis (ECP) remain ones of the most common diseases of the abdominal cavity, multifactorial pathology involving hereditary factors. The genetic studies which have been conducted over the past decades, provided the understanding of the individual components of pathogenic mechanisms of AP and ECP and identified several associated with them candidate genes, such as cationic trypsinogen synthesis (PRSS1), pancreatic secretory trypsin inhibitor (SPINK1), transmembrane regulator protein cystic fibrosis (CFTR), but the mechanisms responsible for the edematous pancreatitis development, are still not fully clarified [1, 2, 3, 4, 5, 6, 7, 8]. The powerful mechanism of the immune system involvement in the pathogenesis of AP and ECP, especially from the positions of the influence of polymorphism of genes which regulate the inflammatory response (interleukin -1β (IL-1β), -4 (IL-4), -6 (IL -6) and tumor necrosis factor-alpha (TNF-α), and others.) on the clinical course of pancreatitis still stays outside the attention of researchers.

The intensity of the inflammatory reaction in response to the etiological factor is individual and depends on the coordinated production, functioning and elimination of numerous cells of biologically active substances, molecules and so on. Extremely important role in the assessing of the inflammatory intensity belongs to cytokines, in particular, IL-1β, TNF-α, C-reactive protein (CRP) and glycoprotein alpha-1-antitrypsin (α1-AT), which is the "acute phase activator". However, these mechanisms are not studied, despite the possible influence of gene *IL-4* (C-590T) polymorphism.

The objective of our investigation was to study some indicators of systemic inflammatory response (TNF-α, IL-1β, CRP and α1-AT) in patients with AP and ECP depending on the gene interleukin 4 (IL-4, (C-590T).

Materials and methods. The study involved 101 patients with AP and ECP (edematous form), who were treated in the Local emergency hospital (Chernivtsi, Ukraine). The diagnosis of AP and ECP was made on the basis of the existing national and European societies' recommendations on the diagnosis and treatment of acute pancreatitis [9, 10]. All the patients signed an informed consent to participate in the research and they underwent a complex of examinations: clinical, laboratory and instrumental ones.

Molecular genetic studies, which included the determining of polymorphic variants of gene *IL-4* (C-590T), were performed at the laboratory of the State institution "Reference centre of molecular diagnostics of the Ministry of Health of Ukraine" (Kyiv) and at the laboratory of Medical Biology and Genetics Department of Bukovinian State Medical University. The polymorphic variants of analysed gene *IL-4* (C-590T) were studied with polymerase chain reaction (PCR) method using oligonucleotide primers of the company "Metabion" (Germany) according to the modified protocols [11]. The amplification products of DNA fragments of gene were further digested with restriction endonuclease ("Thermo Scientific", USA): enzyme AvaII – for gene IL-4. The received fragments were analysed by agarose gel electrophoresis and stained with ethidium bromide, molecular weight marker GeneRuler 50 bp (DNA Ladder, "Thermo Scientific", USA), with further visualization by using transilluminator.

The levels of interleukin IL-1β and the tumor necrosis factor - alpha (TNF-α) were determined by the method of enzyme immuno-enzyme assay (ELISA) using a set of reagents (Interleukin-4 ELISA-BEST) and by chemiluminescence analysis (CLIA) using the Immulite F1427, Siemens. The standard indexes for these parameters were as follows: IL-1β - <5 pg/ml; TNF-α - <8.1 pg/ml.

CRP was determined by photometric analysis method using a set of reagents «Thermo Fisher Scientific» (Finland) and biochemical analyzer KONELAB 20i. The standard index for CRP was level < 0.01 mg/ml. The α1-AT level was determined by nephelometry using the BN ProSpec, Siemens. Standard indexes were 0.9-2.0 g/l.

The correspondence of the genotypes distribution with gene polymorphism to Hardy-Weinberg law in the control group was tested with the chi-square test with 1 degree of freedom, without Yates correction, and the difference in the genotypes distribution in the control group and among the patients - with the chi-square test with 2 degrees of freedom.

The statistical analysis was performed using MYSTAT 12 (Systat Software Inc., USA). The reliability of data for independent samples were calculated by t-test Student (with the distribution of ranges close to normal), or U-criterion Wilcoxon-Mann-Whitney (with uneven distribution). The analysis of qualitative features was performed by the χ^2 criterion. The difference was considered reliable at $p<0.05$.

Results and discussion

Past studies concerning the levels of IL-4 production caused by gene *IL-4* polymorphism, established the lower production of IL-4 in the *TT*-genotype owners of the gene *IL-4* (3.78 pg/ml vs. 31.91 and 19.31 pg/ml of *CC*- and *CT*-genotypes owners, respectively; $p_{CC}<0.001$, $p_{CT}<0.01$).

Determination of TNF-α level in patients with pancreatitis with various levels of IL-4 production caused by gene *IL-4* polymorphism, showed that TNF-α concentrations was higher in the carriers of the wild allele C-590T of gene *IL-4* polymorphism (11.25 and 10.54 pg/ml vs. 4.80 pg/ml of *TT*-genotype owners, respectively, Mann-Whitney criterion - 4.87, $p_{CC}<0.001$, $p_{CT}=0.002$) with no statistically significant difference in the index distribution (Table 1).

Herewith the high production of TNF-α is available in 61.54% of patients with AP with thymine at the 590 promoter position of the gene *IL-4* and in 74.67% of patients with absence of mutations in the selected place ($\chi^2=1.63$; $p>0.05$). We established positive correlation (*S*p-0.140) of weak force (φ-0.145) between the intensity of TNF-α production and low IL-4 production associated with thymine at the 590 promoter position of gene *IL-4* (Table 2).

The use of Clinical Epidemiology methods showed no influence of gene *IL-4* polymorphisms on the TNF-α production level in patients with AP (*RR*-0.624; *95%CI*: 0.357-1.089; Table 3).

Acute disease or exacerbation of chronic inflammation in the pancreas hypothetically ought to be accompanied by the increasing of concentration in blood a major pro-inflammatory cytokines - IL-1β, as a response to factor activity that damages pancreas tissue. Herewith inflammation sanogenesis depends on the balance of pro- and anti-inflammatory cytokines. High concentrations of IL-1β in peripheral blood serum was found in 7.69% of *T*-allele carriers and 34.67% of the owners of *C*-allele of C-590T polymorphism of gene *IL-4* among AP patients (p=0.008; Table 1). We found a positive correlation (*S*p-0.317) of average power (φ-0.213) between the intensity of IL-1β production at low IL-4 production associated with thymine at 590 promoter position of gene *IL-4* (Table 2). The use of clinical

epidemiology methods has allowed to determine that the IL-1β high production is a protective factor for the edematous pancreatitis development in carriers of the *T*-allele of the gene *IL-4* (rs2243250), but increases the risk to the owners of the *C*-allele of the above-mentioned gene (*RR*-0.164; *95% CI*: 0.042-0.647; p<0.05; Table 3).

Table 1

Markers of inflammation depending on the allelic status of *IL-4* gene in patients with edematous pancreatitis

Parameter	Carriers of *T*-allele, n=26 (%)	Carriers of *C*-allele, n=75 (%)	χ^2; p
High production of TNF-α	16 (61.54)	56 (74.67)	χ^2=1.63; p>0.05
High production of IL-1β	2 (7.69)	26 (34.67)	χ^2=7.01; p=0.008
CRP level above 10 mg/ml	16 (61.54)	53 (70.67)	χ^2<1.0; p>0.05
Increasing of α1-AT concentration	9 (34.61)	21 (28.0)	χ^2<1.0; p>0.05

Note. TNF-α – tumor necrosis factor alpha; IL-1β – interleukin 1 beta; α1-AT – alpha-1 antitrypsin; CRP – C-reactive protein.

Table 2

The matrix of correlations between inflammation markers and edematous pancreatitis development in carriers of the mutant *T*-allele of gene *IL-4*

Parameter	Statistical evaluation criteria of the connection between factors			
	*S*p	χ^2	TSFET	φ; connection power
High production of TNF-α	0.140	2.643	0.12756	0.145; weak
High production of IL-1β	0.317	11.128*	0.0000*	0.297; medium
CRP level above 10 mg/ml	0.07	0.651	0.52193	0.072; immaterial
Increasing of α1-AT concentration	0.06	0.392	0.52481	0.056; immaterial

Note. TNF-α – tumor necrosis factor alpha; IL-1β – interleukin 1 beta; α1-AT – alpha-1 antitrypsin; CRP – C-reactive protein; *S*p – Spearman's correlation coefficient; χ^2 - criterion for assessing the significance of the difference of results depending on the risk factor action; TSFET – two-sided Fisher's exact test; φ - the criterion for assessment of the connection power between the risk factor and the result; * - the difference in the indicator distribution is statistically significant (p<0.05).

Table 3

Epidemiological evaluation of inflammatory markers as risk factors of edematous pancreatitis development in carriers of the mutant *T*-allele of gene *IL-4*

Sign	High production of TNF-α	High production of IL-1β	CRP level above 10 mg/ml	Increasing of α1-AT concentration
EER	0.236	0.059	0.256	0.316
CER	0.378	0.359	0.325	0.261
RR	0.624	0.164	0.787	1.208
S (RR)	0.284	0.700	0.293	0.299
95%CI RR	0.357-1.089	0.042-0.647	0.443-1.397	0.673-2.169
Se	0.600	0.057	0.629	0.343
Sp	0.253	0.648	0.297	0.714
OR	0.507	0.112	0.714	1.304
S (OR)	0.421	0.761	0.418	0.425
95%CI OR	0.222-1.158	0.025-0.496	0.314-1.621	0.567-3.000
p	>0.05	<0.05	>0.05	>0.05

Note. *TNF*-α – tumor necrosis factor alpha; *IL-1*β – інтерлейкін 1β; α1-AT – альфа-1 антитрипсин; *CRP* – C-reactive protein; *EER* – experimental event rate; *CER* – control event rate; *RR* – relative risk; *S (RR)* – standard error of the relative risk; *95%CI RR* – 95% confidence interval of the relative risk; *Se* – sensitivity; *Sp* – specificity; *OR* – odds ratio; *S (OR)* – standard error of the odds ratio; *95%CI OR* – 95% confidence interval of the odds ratio.

C-reactive protein (*CRP*) is a protein indicator, which characterizes the acute phase of inflammation. And alpha-1-antitrypsin (α1-AT) is an acute phase activator in pancreas and inhibitor of proteolytic enzymes. The frequency of patients with a higher concentration of *CRP* (>10 mg/ml) and α1-AT between allele carriers of gene *IL-4* (*T*- vs. *C*-allele) did not differ significantly: 61.54% vs. 70.67% (p>0.05) and 34.61% vs. 28.0% (p>0.05), respectively (Table 1). We have not found the correlation between production of CRP (*S*p-0.07; φ-0.072) and α1-AT and AP development depending on *C-590T* genotype of *IL-4* gene (*S*p-0.06; φ-0.056; Table 2) polymorphism.

The use of Clinical Epidemiology methods showed no influence of *C-590T* polymorphism of *IL-4* gene on the CRP and α1-AT production level in patients with AP (*RR*-0.787; *95% CI*: 0.443-1.397; and *RR*-1.208; *95% CI*: 0.673-2.169; Table 3).

Conclusions. 1. The analysis of inflammation process intensity markers in patients with edematous pancreatitis depending on genetically caused production of IL-4 showed that the course of inflammation in the pancreas in dominant homozygotes for the C-590T polymorphism of *IL-4* gene is characterized by increasing concentrations of TNF-α, IL-1β, CRP and IL-4 in peripheral blood serum.

2. The use of Clinical Epidemiology methods helped to prove the role of IL-1β increased production as a risk factor for AP in the *C*-allele carriers of *IL-4* gene selected polymorphism (*RR*=0.164; *OR*=0.112; *95% CI OR*: 0.025-0.50; $p<0.05$) and protective factor of AP development in *T*-allele carriers of *IL-4* gene (rs2243250).

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