THE PARAMETERS OF LIVER FUNCTIONAL STATE AS A RISK FACTOR OF EDEMATOUS Pancreatitis DEVELOPMENT PROVIDING OF GENETIC DETERMINATION OF IL-4 PRODUCTION

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ABSTRACT
Introduction: One of the main pathophysiological mechanisms of acute pancreatitis development is the damage of pancreas cells and hepatocytes with enzymes activation. Recently, a powerful mechanism of the immune system involvement in the acute pancreatitis pathogenesis, in particular, from the position of genes polymorphism influence attracts more attention.

The aim: To study the parameters of liver functional state as risk factors for the development of edematous pancreatitis under the conditions of genetic determination of IL-4 production.

Materials and methods: The study involved 101 patients with acute and the exacerbation of chronic pancreatitis in whom the polymorphic variants of gene IL-4 (C-590T), the activity of ALT, AST, GGTP, LDG and bilirubin fractions levels were determined.

Results: Among the patients with T-allele of IL-4 gene was more commonly encountered the excess of the activity of AST and ALT standards than those with CC-genotype - by 27.94% and 24.33% respectively. The increase of the GGTP concentration was recorded in 79.21% of patients. The serum GGTP level was significantly higher in the TT-genotype owners than in those with C-allele. Hyperbilirubinemia by the total bilirubin, indirect and direct fractions was diagnosed more often in TT-genotype carriers than in CC-homozygotes.

Conclusions: The dysfunction of hepatopancreatobiliary system is more significant in the TT-genotype carriers of IL-4 gene by the AST, ALT, bilirubin and its fractions high levels, however, were found to be risk factors the high levels of total bilirubin and its direct fraction.

KEY WORDS: pancreatitis, gene, IL-4, enzymes, bilirubin

INTRODUCTION
The main pathophysiological mechanisms of acute pancreatitis (AP) development are hypertension of the biliary system, difficulty of outflow, pancreatic secretion hyperproduction, duct hypertension, direct damage of the acinar cells of pancreas and hepatocytes by toxic substances (including alcohol), activation of pancreatic enzymes in ducts and parenchyma, which ultimately leads to autolysis, edema, necrotic changes, and in the future (for a recurrent course) - to the sclerosis and glandular fibrosis with the development of secretory insufficiency [1, 2, 3, 4, 5, 6]. Due to the powerful protective inhibitory mechanisms of counteraction on aggressive pancreatic secretion, the pathological process can be limited to edema without the development of necrosis [7, 8]. However, the hepatobiliary system is also involved in the pathogenetic mechanism of limiting the inflammation pathological process [9]. But, the questions of disintegration of the pancreas and hepatocytes functioning in patients with AP, or the exacerbation of the chronic pancreatitis (ECP), in particular on genetic determination are remain unresolved [10, 11, 12].

Studies aimed at clarifying the role of hereditary factors of edematous AP or ECP, are relevant throughout the world. To date, the registry of hereditary pancreatitis and pancreatic cancer has been even created in Europe [13].

In connection with the above, there was a need to investigate the liver functional state as a risk factor for the development of edematous pancreatitis in the conditions of genetic determination of IL-4 products (rs2243250) to establish its role in the pathogenesis of edematous AP and ECP in order to determine high risk groups, early diagnostics, prognostication and prevention of the illness and its possible complications.

THE AIM
To study the parameters of liver functional state as risk factors for the development of edematous pancreatitis under the conditions of genetic determination of IL-4 production.

MATERIALS AND METHODS
The study involved patients with AP and ECP, admitted to the emergency hospital of Chernivtsi during the last five years. The screening and diagnosis of AP and ECP were carried out according to the current order of the Ministry
of Health of Ukraine [Ministry of Health of Ukraine [14] and the recommendations of the European Societies of diagnosis and treatment of acute pancreatitis [15].

181 patients with edematous form of AP and ECP have passed the screening step, they have signed the informed consent of the patient to participate in the study, with the following complex of clinical-laboratory and diagnostic studies. Genetic studies have being performed on 101 patients, among whom were 19 (18.8%) women and 82 (81.2%) men.

Biochemical studies of the activity of certain cytolysis enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGTP) lactate dehydrogenase (LDH); and consist of bilirubin (total, unconjugated, conjugated) were performed with biochemical analyser KONELAB 20i with the set of reagents «Thermo Fisher Scientific» (Finland). The standard indexes for these enzymes were as follows: AST - men (M) - 35 U/l, women (F) - 31 U/l, ALT - M – up to 45 U/l, F - up to 34 U/l, GGTP - M – up to 55 U/l, F - up to 38 U/l, total bilirubin - <20,5 mcмол/l, conjugated bilirubin - 5,1 mcmol/l, unconjugated bilirubin - <15,4 mcmol/l [16, 17, 18].

Molecular genetic studies, which included the determination of polymorphic variants of gene IL-4 (C-590T), have been performed at the laboratory of the State institution “Centre reference of molecular diagnostics of the Ministry of Health of Ukraine” (Kyiv).

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 [19]. 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 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 ?2 criterion. The difference was considered reliable at p<0.05.

The results of the study were revised by the Biomedical Ethics Commission of the Higher State Educational Institution of Ukraine "Bukovinian State Medical University".

RESULTS AND DISCUSSION

The distribution of genotypes among examined patients was as follows: the gene IL-4 (C-590T) among patients was represented in 58 (57.43%) patients by CC genotype, in 34 (33.66%) - by CT-genotype, in 9 (8.91%) - by mutation TT-genotype.

The increase of aminotransferases concentration in serum is evidence of hepatocytes cytosis and confirmation of the important pathogenetic role of disintegration processes taking place in the hepatobiliary system in the development of active inflammatory process in the pancreas. The increase of the ALT and AST levels was found in 32.67% (n=33) and 65.35% (n=66) of patients with edematous pancreatitis, respectively (Table I): among the patients with «unfavorable» T-allele of IL-4 gene were more commonly encountered the individuals with the excess of the activity of enzymes AST and ALT standarts than those with CC-genotype - by 27.94% (χ²=8.52, p=0.003) and 24.33% (χ²=22.08, p<0.0001) respectively.

However, the correlation analysis (Sp-0.07; φ-0.092; Table II), as well as the methods of clinical epidemiology (RR-0.325; 95% CI: 0.381-1.361; Table III) showed the lack of association between the ALT level in the peripheral blood and C-590T polymorphism of IL-4 gene. And, there is a weak positive relationship (Sp-0.11; φ-0.107) between the AST contents and the edematous AP development in T-allele carriers of IL-4 gene, which, however, was not confirmed as a risk factor for AP occurrence in the examined population (RR -1.412; 95% CI: 0.805-2.474) (Table III).

The increase of the GGTP concentration indicates the presence of intrahepatic cholestasis, as well as, indirectly, the activity of the inflammatory process including the pancreas. The increase of the GGTP concentration was recorded in 79.21% (n=80) of patients with AP. The frequency of the exceeding of the analyzed cholestasis index standart between the genotypes of IL-4 gene trustworthy did not differ (p>0.05; Table I). However, serum GGTP level was significantly higher in the TT-genotype owners than in those with C-allele (p<0.05).

The correlation analysis method revealed a weakly unreliable relationship between the GGTP concentration in blood and the presence of mutation in the 590 position of the promoter of IL-4 gene (Sp-0.07; φ-0.170; Table II). The GGTP concentration increase is associated with C-allele C-590T polymorphism of IL-4 gene by the ratio of chances and risks (RR-0.581), however, the 95% confidence intervals determination did not statistically confirm this assumption (95% CI: 0.333-1.014; Table III).

LDG level, as an indicator of glycolysis activity, tissue respiration and the predictor of hepatocytes necrosis, was elevated in 44 patients with AP: 30.77% of thiamine carriers in 590 position of the promoter of IL-4 gene and 48.0% of the carriers of the wild allele of the selected polymorphism (Table I), without a statistically significant difference in the distribution of the index between the alleles (p>0.05). However, the analysis of individual polymorphic variants showed a higher frequency of excess of LDG standart in CC-genotype owners than those with CT- and TT-variants - by 31.64% (χ²=24.6; p<0.001) and 32.95% (χ²=43.14; p<0.001) respectively. A weak correlation was found between the LDG concentration in blood and the presence of mutation in the 590 position of the promoter of IL-4 gene (Sp-0.153; φ-0.153; Table II). The ratio of chances and risks (RR-0.592) of LDG elevation
was associated with the CC-genotype of C-590T polymorphism of IL-4 gene, however, the determination of 95% confidence intervals did not confirm this indicator as a risk factor for the AP development (95% CI: 0.318 -1.101; Table III).

The determination of the the blood bilirubin fractions in patients with AP allows to analyze the hepatobiliary system work and the protein metabolism activity. Hyperbilirubinemia by the total bilirubin content (within 25-30 mmol/L) was diagnosed in one in five patients with AP: more often in TT-genotype carriers than in CC-homozygotes ($\chi^2$=35.18, $p<0.001$) with respectively a higher indicator - 25.33 versus 14.56 mmol/l (Mann-Whitney's criterion is 2.976; $p=0.022$; Table IV).

Correlation analysis showed a strong feedback between the total bilirubin concentration in blood and the AP

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**Table I.** The liver function parameters depending on the allelic status of IL-4 gene in patients with edematous pancreatitis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients, in general, n=101 (%)</th>
<th>Patients with CC-genotype, n=58</th>
<th>Patients with CT-genotype, n=34</th>
<th>Patients with TT-genotype, n=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>The increasing of AST concentration</td>
<td>66 (65.35)</td>
<td>31 (53.45)</td>
<td>28 (82.35)</td>
<td>7 (77.78)</td>
</tr>
<tr>
<td>The increasing of ALT concentration</td>
<td>33 (32.67)</td>
<td>8 (13.79)</td>
<td>18 (52.94)</td>
<td>7 (77.78)</td>
</tr>
<tr>
<td>The increasing of GGTP concentration</td>
<td>80 (79.21)</td>
<td>46 (79.31)</td>
<td>27 (79.41)</td>
<td>7 (77.78)</td>
</tr>
<tr>
<td>The increasing of LDG concentration</td>
<td>44 (43.56)</td>
<td>32 (55.17)</td>
<td>8 (23.53)</td>
<td>2 (22.22)</td>
</tr>
</tbody>
</table>

Note. AST - aspartate aminotransferase; ALT - alanine aminotransferase; GGTP - gamma-glutamyl transeptidase; LDH - lactate dehydrogenase.

**Table II.** The matrix of correlations between some live function parameters and edematous pancreatitis development in the carriers of the mutant T-allele of gene IL-4

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Statistical evaluation criteria of the connection between parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Sp$</td>
</tr>
<tr>
<td>The increasing of ALT concentration</td>
<td>0.07</td>
</tr>
<tr>
<td>The increasing of AST concentration</td>
<td>0.11</td>
</tr>
<tr>
<td>The increasing of GGTP concentration</td>
<td>0.07</td>
</tr>
<tr>
<td>The increasing of LDG concentration</td>
<td>0.153</td>
</tr>
</tbody>
</table>

Note. AST - aspartate aminotransferase; ALT - alanine aminotransferase; GGTP - gamma-glutamyl transeptidase; LDH - lactate dehydrogenase; $Sp$ – Spearman’s correlation coefficient; $\chi^2$ - criterion for assessing the significance of the difference of results depending on the risk factor action; TSFET – two-sided Fisher’s exact test; $\phi$ - 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 III.** Epidemiological evaluation of some liver function parameters as risk factors of edematous pancreatitis development in the carriers of the mutant T-allele of gene IL-4

<table>
<thead>
<tr>
<th>Sign</th>
<th>The increasing of ALT concentration</th>
<th>The increasing of AST concentration</th>
<th>The increasing of GGTP concentration</th>
<th>The increasing of LDG concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>EER</td>
<td>0.222</td>
<td>0.333</td>
<td>0.218</td>
<td>0.200</td>
</tr>
<tr>
<td>CER</td>
<td>0.309</td>
<td>0.236</td>
<td>0.375</td>
<td>0.338</td>
</tr>
<tr>
<td>RR</td>
<td>0.720</td>
<td>1.412</td>
<td>0.581</td>
<td>0.592</td>
</tr>
<tr>
<td>S (RR)</td>
<td>0.325</td>
<td>0.286</td>
<td>0.284</td>
<td>0.317</td>
</tr>
<tr>
<td>95%CI RR</td>
<td>0.381-1,361</td>
<td>0.805-2.474</td>
<td>0.333-1.014</td>
<td>0.318-1,101</td>
</tr>
<tr>
<td>Se</td>
<td>0.286</td>
<td>0.514</td>
<td>0.486</td>
<td>0.314</td>
</tr>
<tr>
<td>Sp</td>
<td>0.615</td>
<td>0.604</td>
<td>0.330</td>
<td>0.516</td>
</tr>
<tr>
<td>OR</td>
<td>0.640</td>
<td>1.618</td>
<td>0.464</td>
<td>0.490</td>
</tr>
<tr>
<td>S (OR)</td>
<td>0.432</td>
<td>0.400</td>
<td>0.405</td>
<td>0.420</td>
</tr>
<tr>
<td>95%CI OR</td>
<td>0.275-1,492</td>
<td>0.738-3.546</td>
<td>0.210-1.028</td>
<td>0.215-1,116</td>
</tr>
</tbody>
</table>

Note. AST - aspartate aminotransferase; ALT - alanine aminotransferase; GGTP - gamma-glutamyl transeptidase; LDH - lactate dehydrogenase; 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.
in the presence of mutation in the 590 position of the promoter of IL-4 gene (Sp-0.521; ϕ-0.605; Table V). According to the definition of the ratio of chances and risks (RR-7.256), the increase of the total bilirubin concentration is associated with the carrier of the mutant C-590T allele of IL-4 gene polymorphism (95% CI: 3.625-14.526; p<0.05; Table VI).

The concentrations of the indirect and direct bilirubin are also higher in the TT-genotype carriers of C-590T polymorphism of IL-4 gene (12.42 vs. 9.15 mmol/l in the CC-homozygotes of the selected polymorphism and 14.06 mmol/l against 5.41 mmol/l, respectively; the Mann-Whitney criterion is 2.888 and 2.054; p=0.012 and p=0.014, respectively). However, the incidence frequency of high content of indirect and direct bilirubin (n=14 vs. n=31) was not significantly different between genotypes (Table IV).

The correlation analysis method did not establish a significant relationship between the concentration of indirect bilirubin in the blood and the mutation presence of the promoter of IL-4 gene (Sp-0.521; ϕ-0.605; Table V).
ence in the 590 position of the promoter of IL-4 gene (Sp-0.094; φ-0.094; p>0.05; Table V) with the available medium reverse link with the contents of direct bilirubin (Sp-0.174; φ-0.222; p<0.05). The increase of the indirect bilirubin concentration is not associated with the carrier of mutated C-590T polymorphism of IL-4 gene for AP (RR=0.667; 95% CI: 0.143-1.946; p>0.05; Table VI). Instead, the increase of the direct bilirubin content is a risk factor for AP in patients with T-alleles of IL-4 gene (RR=2.029; 95% CI: 1.181-3.488; p<0.05).

This found genotypes distribution of gene IL-4 (C-590T) corresponds to some studies for the healthy [20] but we did not find the data about this gene polymorphism in the patients with acute or chronic pancreatitis.

The bilirubin fractions research as some of specific indicators of cholestasis and toxic influence are the most evident. The level of bilirubin remains close to normal in patients with CC-genotype. But, in general, the carrying of T-allele is combined with the increase of norm indicator and the increase of the bilirubin level in comparison with CC-genotype. We think that the last is caused by besides the toxic alcohol influence, more prominent edematous reaction of the pancreas and of parapancreatic tissue and also by the disfunction of bile outflow [21].

Thus, the AP course in the carriers of the homozygous mutant T-allele of IL-4 gene (C-590T) is associated with the high levels of total bilirubin and its fractions, however, the increased general and direct bilirubin levels are the risk factors. The implementation of acute inflammatory process in the pancreas in T-allele carriers of the same polymorphism variant is characterized by the higher levels of aminotransferases in the serum of peripheral venous blood, which although characterizing the activity of mesenchymal-inflammatory and cholestatic syndromes in the liver, however, did not appear to be the risk factors for the development of the AP.

The obtained results have the scientific novelty because they are received for the first time.

CONCLUSIONS

1. The increased concentration of aminotransferases, GGTP, LDG in blood serum of patients with AP confirmed the presence of mesenchymal-inflammatory, cholestatic and, somewhat less, cytolitic syndromes in the hepatopancreatobiliary system, confirming the presence of its dysfunction (more significant in the TT-genotype carriers of IL-4 gene by the AST and ALT levels – by 27.94% (χ²=8.52, p=0.003) and 24.33% (χ²=22.08, p<0.0001) respectively.

2. The course of AP in the homozygous mutant T-allele carriers of IL-4 gene (C-590T) is associated with the high levels of bilirubin and its fractions, however, were found to be risk factors the high levels of total bilirubin (RR=7.26; OR=20.25; 95% CI OR: 7.57-54.14; p<0.05) and its direct fraction (RR=2.03; OR=2.84; 95% CI OR: 1.23-6.58; p<0.05).

REFERENCES


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Authors’ contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

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