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Research Article

Assessment of Comorbidity as a Risk Factor for Edematous Pancreatitis Considering IL-4 (C-590T) Gene Polymorphism

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Abstract
The comorbidities affect the course of pancreatitis significantly; however, this effect has not been evaluated from the perspective of genetic component.
The objective of the research was to analyse concomitant chronic diseases as potential risk factors for edematous pancreatitis considering genetic predictors.
Materials and methods. The epidemiological analysis of comorbidities (cholelithiasis, urolithiasis, ischemic heart disease, past viral infection) as risk factors for edematous pancreatitis was performed. Polymorphic variants of the IL-4 (rs 2243250) gene being identified in 123 patients and 40 healthy individuals were also involved.
Results. In patients with edematous pancreatitis, urolithiasis was observed more frequently than in the control group - by 37.30% (χ^2=18.62, p<0.001). The presence of cholelithiasis insignificantly increases the risk of edematous pancreatitis in the future (RR-1.156; 95% CI: 0.972-1.374). The presence of urolithiasis increased the risk of edematous pancreatitis twofold (RR-2.039; 95% CI: 1.346-3.090; p<0.05).
Conclusions. 1. Cholelithiasis increases the probability of developing edematous pancreatitis in carriers of the mutant T-allele of the IL-4 gene by 3.7 times (RR=3.69; OR=10.13; 95% CI OR: 3.17-32.42; p<0.05). 2. In the C-allele carriers of the IL-4 (C-590T) gene, the risk of developing acute pancreatitis is twice higher in the presence of urolithiasis or ischemic heart disease, and almost five times higher in case of past viral infection (p<0.05).

Keywords
gene; polymorphism; IL-4; comorbidities

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Problem statement and analysis of the resent research

Numerous medical studies have found more than 200 causes of pancreatitis; alcohol and cholelithiasis have been recognized as the most significant (classificational) ones [1-4, 6-9, 11].

The multisystem manifestations of organ as well as system disintegration being connected with disorders in enterohepatic circulation of free fatty acids, bile passage in the hepatobiliary system, the development of gallstone disease (GSD), cardiovascular diseases (atherosclerosis, ischemic heart disease (IHD), hypertension), urinary disorders (urinary stone disease (USD), inflammatory diseases), metabolic disorders (diabetes mellitus) are prevalent in the pathogenesis of pancreatitis. However, they have not been evaluated from the perspective of genetic component – polymorphisms in candidate genes associated with the development of pancreatitis (PRSS1 (rs111033565), SPINK1 (ID 6690), CFTR (rs113993960)) and genes regulating the inflammatory response to the development of the latter (IL- 4 (rs 2243250), TNF-α (rs1800629)), as additional possible risk factors for acute pancreatitis (AP), and exacerbation of chronic pancreatitis (ECP).

The objective of the research was to analyse concomitant chronic diseases as potential risk factors for edematous pancreatitis considering genetic predictors.

1. Materials and methods

The study involved 123 patients with AP and ECP (edematous form), who were treated in Chernivtsi Regional 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 AP [10, 12]. All the patients signed an informed consent to participate in the research and they underwent a complex of examinations: clinical, laboratory and instrumental ones according to the protocol recommendations. Among the patients there were 23 (18.7%) women and 100 (81.3%) men. The patients' average age was 45.1±5.19 years for males, 53.2±7.07 years for females (from 23 to 77). The control group included 40 apparently healthy individuals of corresponding sex and age.

Molecular genetic studies, which included the determination of polymorphic variants of the IL-4 (C-590T) gene, were performed at the laboratory of the Reference Centre for Molecular Diagnostic, Ministry of Public Health of Ukraine (Kyiv, Ukraine) and the laboratory of the Department of Medical Biology and Genetics of the Bukovinian State Medical University (Chernivtsi, Ukraine). The polymorphic variants of the analysed IL-4 (C-590T) gene were studied using the polymerase chain reaction (PCR) method be means of oligonucleotide primers synthesized in Metabion company (Germany) according to the modified protocols [5]. The amplification products of DNA fragments of the gene were thereafter digested with restriction endonuclease (Thermo Scientific, USA): enzyme AvaII – for the IL-4 gene. The obtained 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 using transilluminator.

The correspondence of the genotype distribution of 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; the difference in the genotype distribution between the control group and the patients was tested using the chi-square test with 2 degrees of freedom.

2. Results and Discussion

The epidemiological analysis of comorbidities, metabolic changes, parameters of the hepato-pancreato-biliary system function, inflammatory markers as risk factors for edematous pancreatitis considering polymorphic variants of the IL-4 (rs 2243250) gene was performed.

The incidence of the most frequent comorbidities as risk factors for edematous pancreatitis is presented in Table 1. In patients with edematous AP, USD occurred significantly more often than in the control group - by 37.30% (χ^2=18.62, p<0.001). Ultrasonography of the liver and biliary tract showed that GSD was found more frequently in patients with AP; however, there was no statistically significant difference in the index distribution (χ^2=1.79; p>0.05).

Atherosclerotic changes in the mesenteric vessels as one of the manifestations of IHD are the triggers of developing trophic changes in the digestive organs including the pancreas and, consequently, the occurrence of inflammation and its chronicity, as well as the development of exocrine pancreatic insufficiency. However, chronic IHD occurred equally often in patients with pancreatitis (36.58%) and in the control group (40.0%, p>0.05; Table 1).

A varied virus flora can be an etiologic factor of inflammation development in the pancreas. The anamnestic indications of past viral infection in the last 6 months were registered in 31.71% of patients with AP and 22.5% of patients of the control group (Table 1), which, however, did not differ statistically significant between the groups (p>0.05).

The Wirsung duct permeability dysfunction being the main cause of pancreatitis, its chronicity and exacerbations

Table 1. Concomitant diseases in patients of the studied groups

Nosology	Main group, n=123 (%)	Control group, n=40 (%)	χ^2; p
GSD	31 (25.20)	6 (15.0)	χ^2=1.79; p>0.05
USD	92 (74.80)	15 (37.50)	χ^2=18.62; p<0.001
IHD	45 (36.58)	16 (40.0)	χ^2<1.0; p>0.05
Viral infection	39 (31.71)	9 (22.50)	χ^2=1.23; p>0.05

Note.
DSG - gallstone disease;
USD - urinary stone disease;
IHD - ischemic heart disease.

development, may be caused by functional or organic changes in the biliary tract. These are often observed in cholelithiasis, which together with urolithiasis may indicate the individual characteristics of mineral metabolism and predisposition to stone formation in hollow organs. The analysis of our epidemiological statistics data showed that GSD presence insignificantly increases the risk of edematous pancreatitis in the future (RR-1.156; 95% CI: 0.972-1.374; Table 2).

The presence of USD increases the risk of edematous pancreatitis twofold (RR-2.039; 95% CI: 1.346-3.090; p<0.05). However, the presence of IHD as well as a positive history of viral diseases (RR-1.446; 95% CI: 0.769-2.719; p>0.05; Table 2) did not increase the probability of AP development (RR-0.936; 95% CI: 0.599-1.462; p>0.05). In our opinion, the peculiarities of the realization of the inflammatory process in the pancreas provided by genetically caused high or low levels of production of basic inflammatory mediators, including IL-4 being one of the main anti-inflammatory interleukins, which, moreover, is associated with T-helper-2-type of inflammatory response are interesting in the scientific aspect.

When determining the anamnestic peculiarities in patients with edematous pancreatitis and genetically caused high/low production of IL-4, GSD was statistically more often observed in carriers of mutant T-allele compared to those with C-allele (by 29.28%) (χ^2=17.54, p¡0.001; Table 3). USD, IHD and past viral infections were significantly more often observed in C-allele carriers compared to owners of T-allele of the IL-4 gene - by 23.64% (χ^2=5.79; p=0.016), 23.44% (χ^2=4.57; p=0.033) and 28.46% (χ^2=7.11; p=0.008), respectively (Table 3). There was found a relatively strong correlation (ϕ=0.417; Table 4) between GSD presence and pancreatitis development in carriers of the T-allele of the IL-4 gene. In addition, GSD increased the risk of edematous pancreatitis approximately by 4 times in patients with the low production of IL-4, caused by the mutation in the promoter region of the IL-4 gene at

position - 590 (RR-3.686; 95% CI: 2.199-6.178; Table 5). There was a medium direct positive correlation between USD and the development of pancreatitis (Sp-0.249), IHD and AP (ϕ-0.213), viral flora and AP (ϕ-0.271) in homozygous carriage of the dominant C-allele of the IL-4 gene (Table 4). USD, IHD and viral infection did not increase the risk of AP appearance in carriers of mutation in the promoter region of the IL-4 gene at position - 590 (RR-0.454; 95% CI: 0.266-0.776), (RR-0.435; 95% CI: 0.206-0.916) and (RR-0.277; 95% CI: 0.105-0.723), respectively (Table 5). Thus, the presence of USD was the risk factor of edematous pancreatitis development, whereas, the presence of cholelithiasis, past viral infection, or atherosclerosis of the vessels with clinical manifestation in the form of IHD did not affect the risk of inflammatory process in the pancreas. Depending on the genetically inherited high or suppressed production of IL-4 it has been found that in the presence of mutant thymine in the promoter region of the IL-4 gene at position - 590 (C-590T) associated with the reduction in the level of coded anti-inflammatory cytokine production, the realization of the inflammatory process in the pancreas is possible if GSD is present without involving other risk factors. Cholelithiasis increased the probability of developing edematous pancreatitis in carriers of the mutant T-allele of the IL-4 gene by 3.7 times.

For clinical manifestation of AP in the owners of dominant cytosine in the promoter region of the IL-4 gene at position - 590 associated with high production of IL-4, the risk factors that reflect vascular and metabolic disorders in the body or are indicative of some etiological factor action are needed. Thus, the presence of USD or IHD increased the risk of AP in the C-allele carriers of the IL-4 (C-590T) gene twofold. Past viral infection increased AP probability in the C-allele carriers of the IL-4 gene by approximately five times.

Table 2. Epidemiological assessment of comorbidities as risk factors for edematous pancreatitis in the investigated population

Sign	GSD	USD	IHD	Viral infection
EER	0.842	0.746	0.365	0.317
CER	0.729	0.366	0.39	0.22
RR	1.156	2.039	0.936	1.446
S (RR)	0.0887	0.212	0.228	0.322
95% CI RR	0.972-1.374	1.346-3.090	0.599-1.462	0.769-2.719
Se	0.254	0.862	0.742	0.816
Sp	0.854	0.448	0.238	0.271
OR	1.986	5.092	1.902	1.654
S (OR)	0.487	0.383	0.419	0.423
95% CI OR	0.765-5.158	2.401-10.795	0.836-4.325	0.722-3.790
p	>0.05	<0.05	>0.05	>0.05

Note.
DSG - gallstone disease;
USD - urinary stone disease;
IHD - ischemic heart disease;
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.

Table 3. Concomitant diseases depending on the allelic state of the IL-4 gene in patients with edematous pancreatitis

Nosology	Carriers of T-allele (52 alleles), n=26 (%)	Carriers of C-allele (150 alleles), n=75 (%)	χ^2; p
GSD	9 (34.61)	4 (5.33)	χ^2=17.54; p<0.001
USD	15 (57.69)	61 (81.33)	χ^2=5.79; p=0.016
IHD	5 (19.23)	32 (42.67)	χ^2=4.57; p=0.033
Viral infection	3 (11.54)	30 (40.0)	χ^2=7.11; p=0.008

Note.
DSG - gallstone disease;
USD - urinary stone disease;
IHD - ischemic heart disease.

3. Conclusions

- In the presence of mutant thymine in the promoter region of the IL-4 gene at position - 590 (C-590T) associated with the reduction in the level of coded anti-inflammatory cytokine production, the realization of the inflammatory process in the pancreas is possible if GSD is present without involving other risk factors.
- Cholelithiasis increases the probability of developing edematous pancreatitis in carriers of the mutant T-allele of the IL-4 gene by 3.7 times (RR=3.69; OR=10.13; 95% CI OR: 3.17-32.42; p¡0.05).
- In the C-allele carriers of the IL-4 (C-590T) gene, the risk of developing acute pancreatitis is twice higher in the presence of USD or IHD, and almost five times higher in case of past viral infection (p<0.05).

4. Prospects for further research

The analysis of inflammatory markers as risk factors for chronic pancreatitis providing genetic determination of IL-4 production is promising.

Table 4. Correlations matrix between indices of comorbidity and edematous pancreatitis development in carriers of T-allele of the IL-4 gene

Factor	Statistical criteria of assessing the relation between factors			
	Sp	χ^2	TSFET	ϕ; connection strength
GSD	-0.311	19.315*	0.00000*	0.417; relatively strong
USD	0.249	7.798*	0.01079*	0.249; average
IHD	0.186	5.697*	0.02251*	0.213; average
Viral infection	0.23	9.232*	0.00248*	0.271; average

Note.
DSG - gallstone disease;
USD - urinary stone disease;
IHD - ischemic heart disease.
Sp - Spearman's correlation coefficient;
χ^2 - criterion for assessing the significance of the difference of the results depending on the risk factor action;
TSFET - two-sided Fisher's exact test;
ϕ - the criterion for assessing the strength of the correlation between the risk factor and the result;
* - the difference in the indicator distribution is statistically significant ($p<0.05$).

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Table 5. Epidemiological assessment of comorbidities as risk factors for edematous pancreatitis in T-allele carriers of the IL-4 gene

Sign	GSD	USD	IHD	Viral infection
EER	0.706	0.213	0.152	0.100
CER	0.191	0.469	0.350	0.360
RR	3.686	0.454	0.435	0.277
S (RR)	0.263	0.273	0.380	0.496
95% CI RR	2.199-6.178	0.266-0.776	0.206-0.916	0.105-0.733
Se	0.400	0.571	0.200	0.114
Sp	0.938	0.187	0.571	0.604
OR	10.133	0.306	0.333	0.197
S (OR)	0.593	0.435	0.473	0.573
95% CI OR	3.167-32.420	0.131-0.718	0.132-0.842	0.064-0.606
p	<0.05	<0.05	<0.05	<0.05

Note.
DSG - gallstone disease;
USD - urinary stone disease;
IHD - ischemic heart disease;
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;
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