УДК 616.37-002-037:575.113

DOI: https://doi.org/10.34287/MMT.1(44).2020.9

Y. V. Avdosiev[1], K. M. Pankiv[2], S. D. Khimich[2], I. V. Belozorov[1], O. M. Kudrevych[1], S. V. Khytruk[2], O. S. Ustimenko[3], M. O. Shostatska[2]
[1]V. N. Karazin Kharkiv National University
Kharkiv, Ukraine
[2]National Pirogov Memorial Medical University
Vinnytsya, Ukraine
[3]Bogomolets National Medical University
Kyiv, Ukraine

Ю. В. Авдосьєв[1], К. М. Паньків[2], С. Д. Хіміч[2], І. В. Белозьоров[1], О. М. Кудревич[1], С. В. Хитрук[2], О. С. Устименко[3], М. О. Шостацька[2]
[1]Харківський національний університет імені В. Н. Каразіна
Харків, Україна
[2]Вінницький національний медичний університет імені М. І. Пирогова
Вінниця, Україна
[3]Національний медичний університет імені О. О. Богомольця
Київ, Україна

THE ROLE OF IDENTIFICATION OF SPINK1 GENE MUTATION STATUS IN PATIENTS WITH ACUTE PANCREATITIS OF ALIMENTARY GENESIS FOR THE PREDICTING THE SEVERITY OF CLINICAL COURSE AND FORMATION OF COMPLICATIONS

Роль ідентифікації мутаційного статусу гена SPINK1 у пацієнтів з гострим панкреатитом аліментарного генезу щодо прогнозування важкості перебігу та формування ускладнень

Abstract

Purpose of the study. *To determine the rate of SPINK1 mutation status in patients with acute pancreatitis of alimentary genesis and to determine the prognostic value of allele status of the gene for the assessment of severity and the formation of complicated course.*

Materials and methods. *70 patients with acute alimentary pancreatitis underwent examination, 48 (68,57%) men and 22 (31,43%) women. Average age made 45,4 ± 13,87 years. Severe course of acute pancreatitis was diagnosed in 34 (48,57 %) persons, in 25 (35,72%) – moderate severity, in 11 (15,71%) – mild cases. Complicated clinical course of acute pancreatitis was recorded in 59 (84,29%) patients of the group, in the rest patients – 11 (15,71%) clinical course had uncomplicated nature. The severity of clinical course and structure of complications were assessed*

Резюме

Мета роботи. *Встановити частоту мутаційного статусу гена SPINK1 у пацієнтів з гострим панкреатитом аліментарного генезу та визначити прогностичну цінність алельного стану гена щодо оцінки ступня важкості та формування ускладненого перебігу.*

Матеріали та методи. *Обстежено 70 хворих з гострим аліментарним панкреатитом, 48 (68,57%) чоловіків та 22 (31,43%) жінок. Середній вік 45,4 ± 13,87 років. Важкий перебіг гострого панкреатиту встановлено у 34 (48,57%) осіб, у 25 (35,72%) – середній, у 11 (15,71%) – легкий. Ускладнений перебіг гострого панкреатиту зафіксовано у 59 (84,29%) хворих групи, у решти – 11 (15,71%) перебіг мав неускладнений характер. Важкість перебігу та структуру ускладнень оцінювали за допомогою класифікації Атланта (2012). Для прогнозування важко-*

according to the classification of Atlanta (2012). In order to predict the severity of acute pancreatitis repeated measurements related to the level of band neutrophils, amylase and glucose were taken. Statistical analysis was performed by means of program STATISTICA (StatSoft Statistica v.10).

Outcomes. *Mutation of SPINK1 gene was statistically found in most cases in patients with acute alimentary pancreatitis with heavy – 16 (47,06%) and moderate severity level – 8 (32,0%) (p = 0,02). The presence of SPINK1 mutation status is related to statistically higher chances of severe clinical course (OR=3,11,CI(1,08–8,92), p = 0,03). In patients with heterozygotic mutations of SPINK1 we have established statistically higher chances for the formation of pancreatic aggregation (OR = 4,5, CI (1,36–14,93), p = 0,01), pseudocysts (OR = 3,58, CI (1,01–12,74), p = 0,04) and pleural empyema (OR = 15,0, CI (1,56–143,83), p = 0,004). The carriers of homozygotic mutations of SPINK1 have higher risks for the development of peritonitis (OR = 12,89, CI (1,01–164,48), p = 0,04), pleurisy (OR = 12,89, CI (1,01–164,48), p = 0,04) and systemic complications (OR = 2,61, CI (2,14–13,14), p = 0,02).*

Conclusion. *Consequently, we have established a high informative value of identification of SPINK1 mutation status in patients with acute pancreatitis of alimentary genesis regarding the prediction of severity level of inflammatory process and the formation of complicated clinical course.*

Keywords: *acute alimentary pancreatitis, SPINK1 mutation status, SPINK1 polymorphism, pancreatitis, gene of pancreatictrypsin inhibitor.*

Результати. *Мутації гена SPINK1 частіше зустрічались у пацієнтів з гострим аліментарним панкреатитом важкого – 16 (47,06%) та середнього ступеня важкості – 8 (32,0%), (p = 0,02). Наявність мутаційного статусу SPINK1 асоційована з достовірно вищими шансами важкого перебігу (OR = 3,11, CI (1,08–8,92), p = 0,03). У пацієнтів з гетерозиготними мутаціями SPINK1 встановлено достовірно вищі шанси формування панкреатичного скупчення (OR = 4,5, CI (1,36–14,93), p = 0,01), псевдокісти (OR = 3,58, CI (1,01–12,74), p = 0,04) та емпієми плеври (OR = 15,0, CI (1,56–143,83), p = 0,004). Носії гомозиготних мутацій SPINK1 мають вищі ризики розвитку перитоніту (OR = 12,89, CI (1,01–164,48), p = 0,04), плевриту (OR = 12,89, CI (1,01–164,48), p = 0,04) та системних ускладнень (OR = 2,61, CI (2,14–13,14), p = 0,02).*

Висновки. *Встановлено високу інформативність ідентифікації мутаційного статусу гена SPINK1 у пацієнтів з гострим панкреатитом аліментарного генезу щодо прогнозування ступеня важкості запального процесу та формування ускладненого перебігу.*

Ключові слова: *гострий аліментарний панкреатит, мутаційний статус гена SPINK1, поліморфізм гена SPINK1, запалення підшлункової залози, ген панкреатичного інгібітору трипсину.*

INTRODUCTION

The problem of diagnosis and management of acute pancreatitis is one of the most complex and relevant in the field of modern surgery of the gastrointestinal tract. In view of the variety and variability of clinical manifestations, early diagnosis of the disease remains problematic regardless the wide range of modern laboratory and testing tools. Besides, the lack of specific therapy is a factor of high incidence of adverse effects of medical management and the formation of a number of comorbid conditions that lower the living standards of persons, preferably of working age.

Multifactorial etiology of acute pancreatitis has been proved. The main factors are ill-balanced diet, excessive consumption of hardened fats, protein deficiency in the diet, starvation, alcohol abuse, etc. The role of genetic factors in the pathogenesis of acute pancreatitis is promising. At present time, a number of mutations have been identified that determine the tendency to acute pancreatitis. Thus, the development activity of autocatalytic process in the pancreas is related to the neutralizing effect of secretory pancreatic trypsin inhibitor, the synthesis of which is encoded by SPINK1 gene. In case of mutation in the pancreatic trypsin inhibitor gene, trypsin inactivation of the gland tissue is impaired, which leads to activation of pancreatic enzymes, proteolytic necrosis of the pancreatic tissues, and venous lysis. The presence of SPINK1 homozygous mutation is related to the processes of intra-acinar trypsin inactivation and is a leading factor in the formation of the inflammatory process in the pancreas.

The importance of genetic factors in the development of the inflammatory process in the pancreas has been described in numerous papers; however the relation of mutational status taking into account the severity of the clinical course and the development of complications remains unstudied. Studying the role of N34S polymorphism of SPINK1 gene in the prediction of severity level and the risk assessment of the complicated clinical course of acute pancreatitis is a pressing problem and requires further study.

RESEARCH OBJECTIVE

To determine the rate of SPINK1 mutation status in patients with acute pancreatitis of alimentary genesis and to determine the prognostic value of allele status of the gene for the assessment of severity level and the formation of complicated clinical course.

MATERIALS AND METHODS

We have analyzed the results of clinical and laboratory examination of 70 patients with acute alimentary pancreatitis who were treated at the unit of surgery of Pyrogov Memorial Vinnytsya Regional Clinical Hospital for the period from 2014 to 2017. The average age of the examined patients was 45,4 ± 13,87 years. The study group included 48 (68,57%) men and 22 (31,43%) women. Diagnostics tools and treatment facilities of patients met the requirements of the unified clinical protocol of rendering medical aid to patients with acute pancreatitis, according to the Order No 297 made by the Ministry of Public Health of Ukraine dated 02.04.2010.

Taking into consideration the severity of clinical course of acute alimentary pancreatitis among the examined patients there were 3 groups. Severe clinical course of acute pancreatitis was diagnosed in the majority of patients in the group – 34 (48,57%), 25 (35,72%) patients had moderate severity, and 11 (15,71 %) patients had mild cases. Taking into account available complications there were two more groups. The complicated clinical course of pancreatitis was recorded in the vast majority – 59 (84,29%) of patients in the group, the rest 11 (15,71%) patients had uncomplicated nature of clinical course. In the structure of complications, local aseptic complications were prevalent, which were observed in 59 (84,29%) patients under study, 35 (50,0%) patients had purulent complications of local nature, and in 3 (4,29%) – local secondary complications. Systemic complications were recorded in 31 (44,29%) patients of the study group. The severity of acute pancreatitis and the presence of complications were assessed using the classification of Atlanta (2012).

SPINK1 gene polymorphism was identified for all the patients in the study group. Genomic DNA was extracted from peripheral blood mononuclear cells using a Gene Jet Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific, USA) according to the manufacturer's instructions. To identify the alleles of SPINK1 gene we used amplification of the appropriate gene segment using the method of allele-specific polymerase chain reaction (two amplification reactions were performed in parallel with two pairs of allele-specific primers) in real time using a set of reagents using the method of SNP-express-PB (Lytech, Russian Federation). Amplification was performed using the device iCycler IQTM5 instrument (Bio-Rad, USA). Amplification mode: 930C, 1 min; 35 cycles: 930C, 10 s.; 640C, 10s, 720C, 20 s.

According to Kolmagorov-Smirnov test, the distribution of the study group differed from normal with a high degree of probability ($p < 0,05$). Non-parametric criteria of Kraskell-Wallis and Mann-Whitney were used to compare the indices of the independent groups. The severity level of acute pancreatitis was predicted on the basis of laboratory indicators using a statistical model of logistic regression. By calculation of odds ratio (OR) and 95% confidence intervals (CI), the relation between the studied laboratory parameters with the severity of acute alimentary pancreatitis was assessed in terms of quantity. Each factor was separately assessed. The probability of an error-free prediction was determined at $p < 0,05$. STATISTICA (StatSoft Statistica v.10) software was used for statistical analysis of the resulting array.

OUTCOMES AND DISCUSSION

Analyzing the rate of SPINK1 mutation status, the presence of heterozygotic and homozygotic mutationswas statistically found in most cases in patients with acute alimentary pancreatitis of heavy severity – 16 (47,06%) and moderate severity level – 8 (32,0%, $p = 0,02$ (table 1). SPINK1 mutation was not found in patients with mild clinical course, 17 (68,0%) patients with acute pancreatitis of moderateseverity leveland 18 (52,94%) persons with severe clinical course. When comparing frequency indices for the absence of mutation status we have established significant difference between the indices ($p = 0,02$). Heterozygotic mutation was recorded in 21 (30,0%) patients of the group – 8 (32,0%) patients with moderate severity level and 13 (38,24%) with severe course, significant difference between the groups was not established ($p = 0,05$). Homozygotic mutation was observed in 3 (8,82%) patients with severe clinical course of inflammatory process, in other groups the given mutation status was not found, difference in mutation rate was statistically insignificant ($p = 0,19$).

The rate of SPINK1 mutation status in patients with complicated clinical course of acute pancreatitis significantly differed from the indices of group with uncomplicated clinical course ($p = 0,03$) (table 2). Mutations of SPINK1 gene were not found in patients with uncomplicated clinical course of acute pancreatitis – 11 (100%) and 35 (59,32%) patients had complications. When comparing frequency indices we have determined significant difference in the indices ($p = 0,03$).

Analyzing the prognostic value of SPINK1 mutation status for assessing the

severity of acute alimentary pancreatitis in patients with homozygous and heterozygous SPINK1mutations, significantly higher chances of severe course (OR = 3,11, CI (1,08–8,92) were identified p = 0,03), instead, the absence of mutation reduces the chances for the development of severe forms (OR = 0,32, CI (0,11–0,92), p = 0,03, (table 3).

Table 1

Characteristics of polymorphic variants of SPINK1 in patients taking into account severe clinical course of acute alimentary pancreatitis

Polymorphic variants	Severity level			p-level
	Mild	Moderate	Heavy	
NN	11 (100,0%)	17 (68,0%)	18 (52,94%)	0,02
NS	0	8 (32,0%)	13 (38,24%)	0,05
SS	0	0	3 (8,82%)	0,19
NS + SS	0	8 (32,0%)	16 (47,06%)	0,02

Table 2

Characteristics of polymorphic variants of SPINK1 in patients taking into account development of complications of acute alimentary pancreatitis

Polymorphic variants	Clinical course		p-level
	Uncomplicated	Complicated	
NN	11 (100%)	35 (59,32%)	0,03
NS	0	21 (35,59%)	0,06
SS	0	3 (5,09%)	0,80
NS + SS	0	24 (40,68%)	0,03

Table 3

Prediction of severe clinical course of acute alimentary pancreatitis in patients with SPINK1 polymorphic variants

Genetic status	Severity level					
	Mild		Moderate		Heavy	
	OR	p-level	OR	p-level	OR	p-level
NN		0,001		0,76	0,32 (0,11–0,92)	0,03
NS		0,003		0,79		0,14
SS		0,31		0,10		0,03
NS, SS		0,001		0,76	3,11 (1,08–8,92)	0,03

Analyzing the risk of complicated course in patients with heterozygous SPINK1 mutations it was established that there are significantly higher chances for the formation of pancreatic aggregate (OR = 4,5, CI (1,36–14,93), p = 0,01) and pseudocysts (OR = 3,58, CI (1,01–12,74), p = 0,04 (table 4). Carriers of homozygous SPINK1 mutations have a higher risk for the development of peritonitis (OR = 12,89, CI (1,01–164,48), p = 0,04) and pleurisy (OR = 12,89, CI (1,01–164,48), p = 0,04). The absence of SPINK1 mutations is related to a reduction in the risk of progression of peritonitis (OR = 0,23, CI (0,06–0,92), p = 0,03), pleurisy (OR = 0,23, CI (0,06–0,92), p = 0,03), pancreatic aggregate (OR = 0,10, CI (0,03–0,36), p = 0,0001), pseudocyst formation (OR = 0,24, CI (0,07–0,88), p = 0,03) and pancreatogenic diabetes (OR = 0,08, CI (0,009–0,80), p = 0,009).

Analyzing the risk of development of purulent local complications in patients with SPINK1 heterozygotic mutationit was determined that there are higher chances for the formation of pleural empyema (OR = 15,0, CI (1,56–143,83), p = 0,004), and the absence of mutation reduces the risk of purulent peritonitis (OR = 0,30, CI (0,09–0,97), p = 0,04), pleural empyema (OR = 0,08, CI (0,009–0,80), p = 0,009), phlegmon (OR = 0,23, CI (0,06–0,92), p = 0,03) and purulent pseudocyst (OR = 0,21, CI (0,05–0,96), p = 0,03 (table 5).

Table 4

Overall risk of complications and risk of local aseptic complications of acute alimentary pancreatitis taking into account SPINK1 polymorphic variants

Complications	Polymorphic variants					
	NN		NS		SS	
	OR	p-level	OR	p-level	OR	p-level
General		0,001		0,003		0,31
Local		0,001		0,003		0,31
Peritonitis	0,23 (0,06–0,92)	0,03		0,24	12,89 (1,01–164,48)	0,04
Pleurisy	0,23 (0,06–0,92)	0,03		0,24	12,89 (1,01–164,48)	0,04
Pancreatic aggregate	0,10 (0,03–0,36)	0,000	4,5 (1,36–14,93)	0,01*		0,002
Acute necrosis		0,001		0,003		0,31
Pseudocyst	0,24 (0,07–0,88)	0,03	3,58 (1,01–12,74)	0,04		0,53
Pancreatogenic diabetes	0,08 (0,009–0,80)	0,009	5,53 (0,90–34,06)	0,05		0,21

Table 5

The risk of development of local purulent complications of acute pancreatitis in patients taking into account SPINK1 polymorphic variants

Complications	Polymorphic variants					
	NN		NS		SS	
	OR	p-level	OR	p-level	OR	p-level
Infectious aggregates		0,33		0,63		0,30
Circumscribed necrosis		1,0		1,0		1,0
Purulent peritonitis	0,30 (0,09–0,97)	0,04		0,18		0,10
Abscesses		0,56		0,34		0,28
Pleural empyema	0,08 (0,009–0,80)	0,000	15 (1,56–143,83)	0,004		0,46
Phlegmon	0,23 0,06–0,92	0,03		0,06		0,44
Purulent pseudocyst	0,21 (0,05–0,96)	0,03		0,09		0,35

A higher risk for the formation of local secondary complications of acute alimentary pancreatitis taking into account SPINK1 polymorphic variants was not determined in the patients under study (table 6).

Table 6

The risk of development of localsecondary complications of necrotic pancreatitis in patients taking into account SPINK1 polymorphic variants

Complications	Polymorphic variants					
	NN		NS		SS	
	OR	p-level	OR	p-level	OR	p-level
Acute bleeding		0,14		0,12		0,77
Fistulas		0,19		0,23		0,67
Arrosive hemorrhage		1,0		1,0		1,0

It was determined that patients with SPINK1 homozygous mutations had a significantly higher chance of developing systemic complications (OR = 2,61, CI (2,14–13,14), p = 0,02 (instead we

have proved that the absence of mutation reduced the chances of developing systemic inflammation response syndrome (OR = 0,29, CI (0,10–0,87), p = 0,02) and systemic complications as a whole (OR = 0,24, CI (0,08–0,70), p = 0,006 (table 7).

Table 7

The risk for development of systemic complications of acute pancreatitis in patients taking into account SPINK1 polymorphic variants

Complications	Polymorphic variants					
	NN		NS		SS	
	OR	p-level	OR	p-level	OR	p-level
Systemic	0,24 (0,08–0,70)	0,006	2.80 (0,96–8,19)	0,05	2,61 (2,14–13,14)	0,02
Systemic Inflammation Response Syndrome	0,29 (0,10–0,87)	0,02		0,09		0,16
Organ Failure Syndrome	0,17 (0,03–1,00)	0,03		0,11		0,26
Multiple Organ Dysfunction Syndrome		0,11		0,17		0,53

CONCLUSIONS

We have recorded a significantly higher rate of SPINK1 mutation status identification in patients with severe forms of acute alimentary pancreatitis and in case of a complicated course.

We have proved a high prognostic value of SPINK1 mutation status identification for assessing the severity of the inflammatory process of the pancreas.

We have determined that the identification of SPINK1 mutation status is a reliable and accurate criterion for assessing the risk of complications in case of acute pancreatitis of alimentary genesis.

REFERENCE

1. Borowitz MJ, Wood BL, Devidas M et al.Prognostic significance of minimal residual disease in high risk B-ALL: A report from Children's Oncology Group study AALL0232. Blood. 2015; 126 (8): 964–971. DOI: 10.1182/blood-2015-03-633685.

2. Greenberg JA, Hsu J, Bawazeer M et al. Clinical practice guideline: management of acute pancreatitis. Can J Surg. 2016; 59 (2): 128–140. DOI: 10.1503/cjs.015015.

3. Karakayali F. Surgical and interventional management of complications caused by acute pancreatitis. World J Gastroenterol. 2014; 20 (37): 13412–13423. DOI: 10.3748/wjg.v20.i37.13412.

4. Mounzer R, Whitcomb DC. Genetics of acute and chronic pancreatitis. Curr Opin Gastroenterol. 2013; 29 (5): 544–551. DOI: 10.1097/MOG.0b013e3283639383.

5. Shah AP, Mourad MM, Bramhall SR. Acute pancreatitis: current perspectives on diagnosis and management. Journal of inflammation research. 2018; 11: 77–85. DOI: 10.2147/JIR.S135751.

6. Werner J, Uhl S, Feuerbach W, Buchler M. Management of acute pancreatitis: from surgery to interventional intensive care. Gut. 2005; 54 (3): 426–436. DOI: 10.1136/gut.2003.035907.

7. Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology. 2013; 144: 1252–1261. DOI: 10.1053/j.gastro.2013.01.068.

Стаття надійшла до редакції 18.01.2020