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I.V. Karol, S.V. Ziablitsev[1], O.O. Bilyayeva
Shupyk National Healthcare University of Ukraine, Kyiv
[1]Bogomolets National Medical University, Kyiv

ACUTE PERITONITIS AND POSSIBLE CONNECTION WITH 5,10-METHYLENETETRAHYDROFOLATEREDUCTASE GENE POLYMORPHISMS rs1801133 AND rs1801131

e-mail: drkarol@ukr.net

The purpose of the study was to analyze the condition of patients with acute peritonitis and to establish the connection with polymorphisms of the 5,10-methylenetetrahydrofolate reductase gene rs1801133 and rs1801131. 100 patients who were operated on for peritonitis were divided into three groups by diagnosis: 1st – acute appendicitis (48 %), 2nd – acute cholecystitis (32 %) and 3rd – other diseases (22 %), including perforated stomach ulcer or duodenal (8 %), tumour perforation (3 %), abdominal trauma (3 %), pinched hernia (2 %), and intestinal obstruction (4 %). 50 people without such pathology were involved for control. The allelic polymorphism rs1801133 of the 5,10-methylenetetrahydrofolate reductase gene was connected with the development of peritonitis (except for patients with acute appendicitis): mutant allele A increased the risk of peritonitis compared to controls (OR 1.9; 95 % PI 1.06-3.49; p=0.038). The allelic polymorphism rs1801131 of the 5,10-methylenetetrahydrofolate reductase gene increased the risk of peritonitis in patients with acute appendicitis compared with all other diagnoses (OR 2.03; 95 % PI 1.07-3.84; p=0.039). The results showed that the risk of peritonitis depended on the presence of polymorphisms rs1801133 and rs1801131 of the 5,10-methylenetetrahydrofolate reductase gene.

Key words: peritonitis, 5,10-methylenetetrahydrofolate reductase, rs1801133, rs1801131, multiple organ failure.

І.В. Кароль, С.В. Зябліцев, О.О. Біляєва

ГОСТРИЙ ПЕРИТОНІТ ТА МОЖЛИВИЙ ЗВ’ЯЗОК З ПОЛІМОРФІЗМАМИ ГЕНА 5,10-МЕТИЛЕНТЕТРАГІДРОФОЛАТРЕДУКТАЗИ rs1801133 ТА rs1801131

Метою дослідження було проведення аналізу стану пацієнтів з гострим перитонітом та встановлення зв’язку з поліморфізмами гена 5,10-метилентетрагідрофолатредуктази rs1801133 і rs1801131. 100 пацієнтів, які були прооперовані з приводу перитоніту, за діагнозом було розподілено на три групи: 1-а – гострий апендицит (48 %), 2-а – гострий холецистит (32 %) і 3-я – інші захворювання (22 %), у тому числі, перфоративна виразка шлунку або дванадцятипалої кишки (8 %), перфорація пухлини (3 %), травми черевної порожнини (3 %), защемлена грижа (2 %) та кишкова непрохідність (4 %). У якості контролю залучено 50 осіб без такої патології. Алельний поліморфізм rs1801133 гена 5,10-метилентетрагідрофолатредуктази мав зв’язок з розвитком перитоніту (за винятком пацієнтів з гострим апендицитом): мутантна алель *A* збільшувала ризик перитоніту у порівнянні з контролем (ВШ 1,9; 95 % ВІ 1,06-3,49; p=0,038). Алельний поліморфізм rs1801131 гена 5,10-метилентетрагідрофолатредуктази збільшував ризик перитоніту у пацієнтів з гострим апендицитом у порівнянні зі всіма іншими діагнозами (ВШ 2,03; 95 % ВІ 1,07-3,84; p=0,039). Результати роботи показали, що ризик перитоніту залежав від наявності поліморфізмів rs1801133 і rs1801131 гена 5,10-метилентетрагідрофолатредуктази.

Ключові слова: перитоніт, 5,10-метилентетрагідрофолатредуктази, rs1801133, rs1801131, поліорганна недостатність.

The study is a fragment of the research project “Optimization of diagnosis and treatment of patients with general and urgent surgical pathology”, state registration No. 0120U100927.

Nowadays peritonitis is one of the most serious complications of acute diseases of the abdominal cavity. The widespread use in clinical practice of computed tomography, endoscopic and laparoscopic techniques for the diagnosis and treatment of peritonitis, the use of modern approaches in anesthesiology and intensive care, methods of detoxification of the body does not provide significant progress in the treatment of widespread peritonitis [1]. Therefore, according to various authors, the mortality rate in peritonitis with the development of abdominal sepsis and multiple organ failure ranges from 19 to 80 % [2]. The pathogenesis of peritonitis is a complex process of the interaction of an infectious agent, the body's pathological reactions to the inflammatory process, and the body's defences. A symptom complex that includes pain, inflammation, violation of the integrity of the abdominal organs, intestinal paresis, disturbances in acid-base and water-electrolyte metabolism, intoxication, disturbances in microcirculation and hemodynamics, tissue respiration, immunosuppression, and the development of multiple organ failure occurs with peritonitis [8].

The nature of the exudate and the prevalence of peritonitis, which causes complications from the internal organs, are the most important factors affecting the course of the postoperative period. It has also been established that the time of surgical intervention is the main factor determining mortality in patients with peritonitis [3]. Therefore, the latest methods of studying peritonitis are an important aspect in the search for new therapeutic and diagnostic algorithms. Such methods include genetic research.

The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) is a catalyst of the folate metabolism pathway which products are involved in the remethylation of homocysteine to methionine. Methionine is the precursor of the main methyl donor for DNA methylation and gene regulation. Two polymorphic variants of the MTHFR gene: rs1801133 (C677T) and rs1801131 (A1298C) cause a mild form of MTHFR deficiency, which is associated with the risk of cardiovascular diseases due to hyperhomocysteinemia and is associated with recurrent pregnancy loss, neural tube defects and congenital anomalies, cancer and other diseases [9]. It is important to explore the connection of this gene with the development of various forms of peritonitis.

So, it follows from the above that this research is relevant today and requires in-depth study to improve the effectiveness of treatment of patients with peritonitis.

The purpose of the study was to analyze the condition of patients with acute peritonitis and establishing a connection with polymorphisms of the 5,10-methylenetetrahydrofolate reductase gene rs1801133 and rs1801131.

Materials and methods. The study involved 100 patients who were operated on for acute abdominal diseases complicated by peritonitis. Depending on the diagnosis, patients were divided into three groups: 1st – acute appendicitis (48 %), 2nd – acute cholecystitis (32 %) and 3rd – other diseases (22 %), including perforated ulcer stomach or duodenum (8 %), tumor perforation (3 %), abdominal trauma (3 %), incarcerated hernia (2 %) and intestinal obstruction (4 %). 50 people without such pathology were involved as a control. The World Medical Association's Declaration of Helsinki on Ethical Principles of Medical Research with Human Subjects (2000) was followed during the research.

The MTHFR gene is located on chromosome 1 (1p36.22, OMIM 607093, NM_005957). Its two most common polymorphisms are rs1801133 (C 677T; c.665C>T; p.Ala222Val) and rs1801131 (A 1298C; c.1286A>C; p.Glu429Ala) [14]. Genotypes of MTHFR gene polymorphisms were determined by real-time polymerase chain reaction using the Gene Amp® PCR System 7500 amplifier (Applied Biosystems, USA). Genomic DNA was isolated from venous blood (PureLink® Genomic DNA Kit For Purification of Genomic DNA; “INVITROGEN”; USA). TaqMan Mutation Detection Assays Life-Technology (USA) test systems were used for genetic analysis.

Statistical processing of the research results was carried out using the EZR v.1.54 program (graphical user interface for R statistical software version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria) [6]. The Shapiro-Wilk test was used to check the distribution of quantitative indicators for normality. The distribution law differed from the normal one, so the median (Me) and first and third quartiles (Q1-Q3) were calculated for presentation; Mann-Whitney, Wilcoxon and Kruskal-Wallis rank variance analysis tests were used to compare samples. The difference in the frequency of genotypes and alleles was compared by Fisher's exact method and Pearson's $\chi 2$ test. The statistical probability of differences in the frequency distribution of genotypes and alleles "case-control" was evaluated in conjugation tables (3×2 and 2×2, respectively). The degree of association of genotypes and alleles with PVCG was determined by calculating the odds ratio (OR) and 95 % probability interval (95 % CI). Values of $p<0.05$ were considered probable in all cases of statistical evaluation.

Results of the study and their discussion. Analysis of clinical data showed that the age of patients was from 18 to 91 years (43; 32.0–59.5), 55 % were women and 45 % were men. The distribution of patients in groups by gender (Fig. 1a) showed a significant increase in the proportion of women in the 2nd group (81.2 %; p=0.001).

According to the prevalence of the process, the majority of patients (70 %) had local peritonitis and the remaining patients (30 %) had widespread forms (diffuse – 18 %; spilled – 7 %; general – 5 %). Stratification of patients by the group revealed that the majority of patients of the 1st group (77.1 %) and all patients of the 2nd group have local peritonitis (see Fig. 1b), and the majority of patients (95 %) of the 3rd group had widespread forms of peritonitis (diffuse – 35 %; spilled – 35 %; general – 25 %).

The serous or serous-fibrinous exudate was determined in 70 % of patients, in the remaining cases, there was fibrinous-purulent (11 %), purulent (14 %), faecal (3 %) and hemorrhagic (2 %) exudate. The majority of patients in all groups (see Fig. 1c) had a serous or serous-fibrinous exudate (62.4 % in the 1st, 90.6 % in the 2nd, and 55.0 % in the 3rd). Fibrinous-purulent or purulent (35.4 % of cases) and hemorrhagic (2.1 %) exudate was also detected in the 1st group. In the 2nd group, the exudate was purulent in 9.4 % of cases, and in the 3rd group, it was fibrinous-purulent (25 %), faecal (15 %), and hemorrhagic (5 %) exudate.

The majority of patients (58 %) had the toxic stage of peritonitis, 37 % of patients had the reactive stage and 5 % of patients had the terminal stage of peritonitis. An approximately equal distribution of

patients between reactive and toxic stages (52.1 % and 47.9 %, respectively) was found in the 1st group (see Fig. 1d), while in the 2nd group, the toxic stage was more often determined than the reactive stage (75 % and 25 %, respectively). In the 3rd group, the toxic stage of peritonitis was more frequent (55 %), the reactive and terminal stages were determined in 20 % and 25 % of cases, respectively.

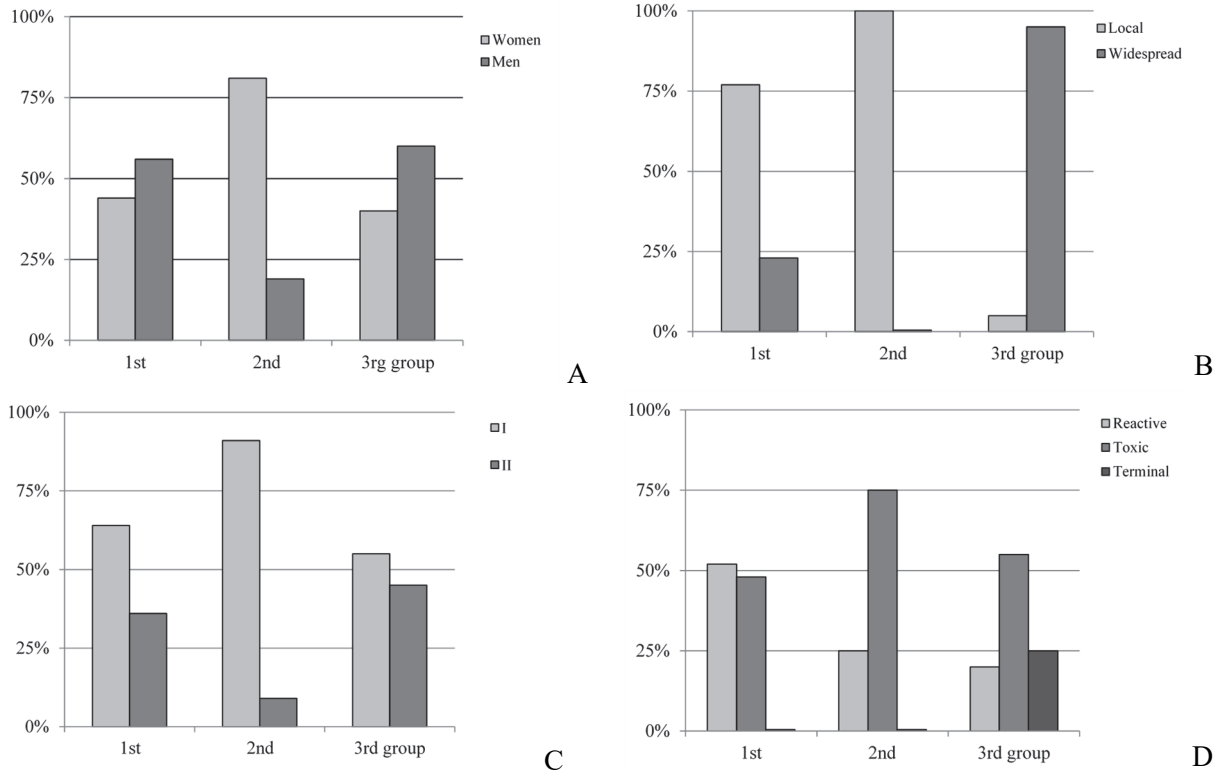

Fig. 1. Characteristics of clinical data by the group; a – distribution by gender (p=0.001); b – distribution according to the prevalence of the process (p<0.001); c – distribution by nature of exudate (I – serous and serous-fibrinous, II – purulent, faecal and hemorrhagic; p<0.001); d – distribution by stage of peritonitis (p<0.001).

The majority of patients with peritonitis had II blood group (42 %), 27 % had I group, III – 22 %, and IV – 9 % (p=0.01).

The median number of bed days was 8 days (7–10), the duration of the operation was 70 minutes (50–100), the temperature during hospitalization was 37.2°C (37–37.5°C), normalization of the temperature occurred on the 4th day (3–5).

Patients of the 1st group were younger than those in the other groups by 17.5–18 years (p=0.001). The number of bed days increased significantly in the groups with a maximum (11 days) in the 3rd group (p<0.001). Also, the duration of the operation was the longest in the 3rd group (p<0.001). In addition, normalization of body temperature in the 3rd group occurred later (on the 5th day) than in the other groups (p=0.01). According to hemodynamics and breathing parameters, there was no significant difference between the groups.

A separate analysis based on the prevalence of peritonitis showed that the number of bed days (p=0.033) and the duration of surgery (p=0.003) were significantly higher in patients with widespread peritonitis in comparison with local peritonitis. There was no statistically significant difference for other indicators (p>0.05; not shown in the table).

It was also found that local peritonitis was predominantly serous or serous-fibrinous in 75.7 % of cases, while widespread peritonitis was so in only 56.6 % (p<0.001). Patients with serous or serous-fibrinous exudate had significantly lower age (by 10 years; p=0.45), the number of bed days (p=0.01) and duration of surgery (p=0.45). Also, their body temperature recovered faster (p<0.001).

The frequencies of genotypes and alleles of polymorphisms rs1801133 and rs1801131 of the MTHFR gene in patients with peritonitis and the control group did not differ statistically significantly from each other (Fig. 2).

The Hardy-Weinberg test for cases and controls showed random inheritance for both polymorphisms (p>0.05). The calculation of conjugation tables for genotypes (3×3) and alleles (2×2) did not show the statistical significance of their distribution in patients with peritonitis in comparison with the control group for both polymorphisms (p>0.1) and, accordingly, the existence of their association with disease.

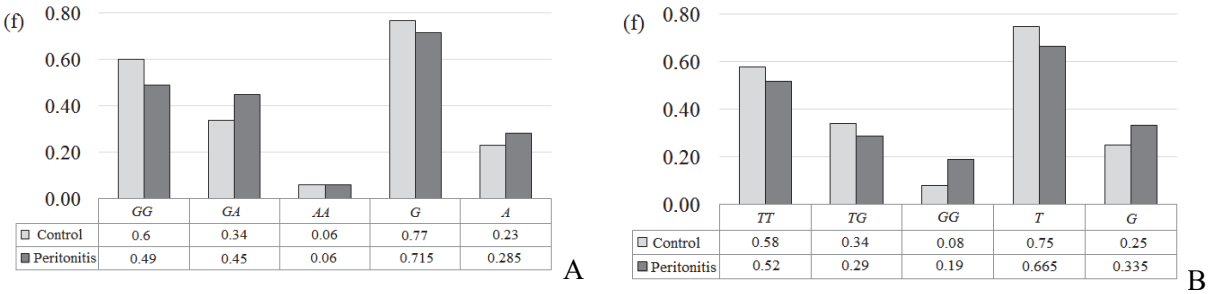

Fig. 2. Frequency distribution of genotypes (p=0.419) and alleles (p=0.335) of rs1801133 of the MTHFR gene (a) and genotypes (p=0.210) and alleles (p=0.145) of rs1801131 of the MTHFR gene (b) in patients with peritonitis and in control group.

Within the framework of this study, the validity of the distribution of genotypes and alleles and their relationship with the disease according to both polymorphisms was verified by separately comparing each group with the control, all groups with each other, as well as by prevalence (local and widespread peritonitis) and by the nature of the exudate. Statistically significant differences were obtained for rs1801133 - for the distribution of alleles when comparing patients of the 2nd and 3rd groups with controls (Table 1) and for rs1801131 - for the distribution of alleles in patients of the 1st group in comparison with the 2nd and 3rd groups (Table 2).

Table 1

Frequency of the rs1801133 alleles' polymorphism of the *MTHFR* gene and the effect on the peritonitis development in patients of the 2nd and 3rd groups

Alleles	Groups		p	OR	95 % PI
	2nd + 3rd, n (f)	Control, n (f)			
G	75 (0.636)	77 (0.770)	0.038	0.52	0.29–0.95
A	43 (0.364)	23 (0.230)		1.92	1.06–3.49

Notes: f – the allele frequency; p – the probability of differences with the null hypothesis according to Fisher's exact test; OR – odds ratio; 95 % PI – a 95 % probable interval for OR.

Table 2

Frequency of the rs1801131 alleles polymorphism of the *MTHFR* gene and the impact on the peritonitis development in patients with acute appendicitis (group 1) compared to other patients

Alleles	Groups		p	OR	95 % PI
	1st, n (f)	2nd + 3rd, n (f)			
T	60 (0.625)	71 (0.771)	0.039	0.49	0.26–0.93
G	36 (0.375)	21 (0.228)		2.03	1.07–3.84

Notes: f – the allele frequency; p – the probability of differences with the null hypothesis according to Fisher's exact test; OR – odds ratio; 95 % PI – a 95 % probable interval for OR.

Analysis of conjugation tables (2×2) showed that the allelic polymorphism of rs1801133 was associated with the development of peritonitis (but not for patients with acute appendicitis): the minor allele A increased the chances of developing peritonitis (OR=1.9; 95 % PI 1.06–3.49; p=0.038).

The allelic polymorphism of rs1801131 also showed a connection with the development of peritonitis, but this was manifested only for carriers of the mutant allele G, which increased the risk of peritonitis in acute appendicitis in comparison with all other diagnoses (OR=2.03; 95 % PI 1.07–3.84; p=0.039).

According to large retrospective analysis, the average age of patients with peritonitis was 58±19 years with a gender ratio of 1.08 [10], which responded to our data, except for patients with acute cholecystitis, where the proportion of women was 81.2 %. In our opinion, this is related to the higher frequency of cholecystitis in women. Peritonitis had a purulent character in 52.9 % of cases, [10], while in our study such exudate occurred in 14 % of cases.

MTHFR is a key enzyme in the metabolism of folic acid and homocysteine, it irreversibly converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the methyl donor for the methylation of homocysteine to methionine [9]. The two most common polymorphisms of the *MTHFR* gene – 677C>T and 1298A>C are common in 30–50 % of the general population and are associated with an increased risk of many diseases: diabetes [15], risk of vascular disasters [11], arterial hypertension [5], malformations [13], oncological pathology [12], as well as diseases associated with chronic inflammation - psoriasis, multiple sclerosis and inflammatory bowel diseases [4].

Today, the mechanism underlying the association between *MTHFR* gene polymorphisms and diseases is not well understood [7]. In this regard, most studies have focused on elevated homocysteine levels and folic acid metabolism. However, systemic inflammation is known as a key factor in the

pathogenesis of not only chronic inflammatory diseases but also many other diseases, including diabetes, coronary disease, cancer, etc. For example, diabetic patients with mutant homozygous (*TT*) *MTHFR C677T* (rs1801133) have elevated levels of C-reactive protein and pro-inflammatory interleukin-6 [7].

Therefore, the tendency to develop chronic systemic inflammation may be one of the main pathophysiological mechanisms of the effect of *MTHFR* gene polymorphisms in surgical patients. In this regard, our study showed that the risk of more clinically severe variants of peritonitis was significantly associated with carrying the mutant allele G of the rs1801133 polymorphism of the MTHFR gene. It was also established that the risk of peritonitis was increased by 2 times in carriers of the *G* rs1801131 mutant allele who had acute appendicitis compared to other patients (p=0.039).

Conclusions

1. The majority of patients with acute appendicitis (77.1 %) and cholecystitis (100 %) had localized peritonitis, whereas 95 % of patients with perforated ulcer, tumour perforation, abdominal trauma, incarcerated hernia, and intestinal obstruction had disseminated peritonitis. Most patients had a serous or serous-fibrinous exudate (70 %) and a toxic stage of peritonitis (58 %). II blood group was determined (42 %) in the majority of patients with peritonitis.

2. Local peritonitis was predominantly serous or serous-fibrinous in 75.7 % of cases and only 56.6 % of patients had widespread peritonitis (p<0.001). Patients with serous or serous-fibrinous exudate had a significantly lower age (by 10 years; p=0.45), the number of bed days (p=0.01) and duration of surgery (p=0.45); their body temperature recovered faster (p<0.001).

3. The allelic polymorphism rs1801133 of the *MTHFR* gene was associated with the development of peritonitis (except for patients with acute appendicitis): the mutant allele *A* increased the risk of peritonitis compared to the control (OR 1.9; 95 % PI 1.06–3.49; p =0.038).

4. The allelic polymorphism rs1801131 of the *MTHFR* gene increased the risk of peritonitis in patients with acute appendicitis compared to all other diagnoses (OR 2.03; 95 % PI 1.07–3.84; p=0.039).

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