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POLYMORPHISM OF MATRIX METALLOPROTEINASE-2 (C^{-1306}→T) GENE IN PATIENTS WITH INTESTINAL ANASTOMOTIC LEAK IN UKRAINIAN POPULATION

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Introductions. One of the most urgent problems of abdominal surgery is intestinal anastomotic leak. The incidence of such complications, according to various authors, ranges from 2-8,1% in small intestinal anastomosis, to 3,8-14,6% in operations on the colon [1]. Anastomotic leak is accompanied by the mortality rate of 14-21,7% [2], with the development of disseminated peritonitis, abdominal sepsis, mortality increases up to 43-82,9% [3]. So far, there is no single point of view in the surgical community regarding the causes of anastomotic leak development and surgical tactics in the development of these complications.

Although there is no doubt about the role of regenerative processes in the formation of intestinal anastomosis [4], scientific publications and research at the current methodological level on this topic are not enough.

Given the almost unexplored role of genetic predisposition in the development of postoperative complications, namely the anastomotic leak, our goal is to study the polymorphism of genes encoding matrix metalloproteinase-2 (MMP-2).

Aim. To analyze the frequency of polymorphic variants of gene MMP-2 (C^{-1306}→T) in patients with intestinal anastomotic leak.

Materials and methods. A prospective trial was based on data from 17 patients, who were treated in the Shalimov National Institute of Surgery and Transplantology during 2017-2020. All patients suffered intestinal anastomotic leak. For the assessment of genetic polymorphism in the population, 80 practically healthy people matched by gender and age with the experimental group were examined.

Genetic studies were performed in the laboratory of the department of general and molecular pathophysiology at the Bogomoletz Institute of Physiology NAS of Ukraine. Buccal epithelium was collected using buccal brushes followed by freezing of the samples and storing them at -20° C. DNA for the genotyping was extracted from the samples using DiatomTM Prep 200 («Isogen Laboratory», RF) according to the manufacturer's protocol.

The following polymorphisms were studied by real-time PCR: C^{-1306}→T (MMP2), rs243865. Amplification reactions were performed using the Fast Real-time PCR System (Applied Biosystems, USA) in a final reaction volume of 20 μl containing 2X TaqMan Universal Master Mix (Applied Biosystems, USA), assay C_1792560_10 and template DNA. Amplification of gene fragments consisted of a denaturation step at 95° C for 20 sec, followed by 40 cycles of amplification at 95° C for 3 sec and 60° C for 30 sec. Data analysis was performed with 7500 Fast Real-Time PCR Software (Applied Biosystems, Foster City, USA).

Results and discussion. To identify the possible association of polymorphic variants of the MMP-2 (C^{-1306}→T) gene with the risk of anastomotic leak, we performed a one-way analysis of variance of the frequency of genotypes in the studied groups of patients (table 1).

Table 1

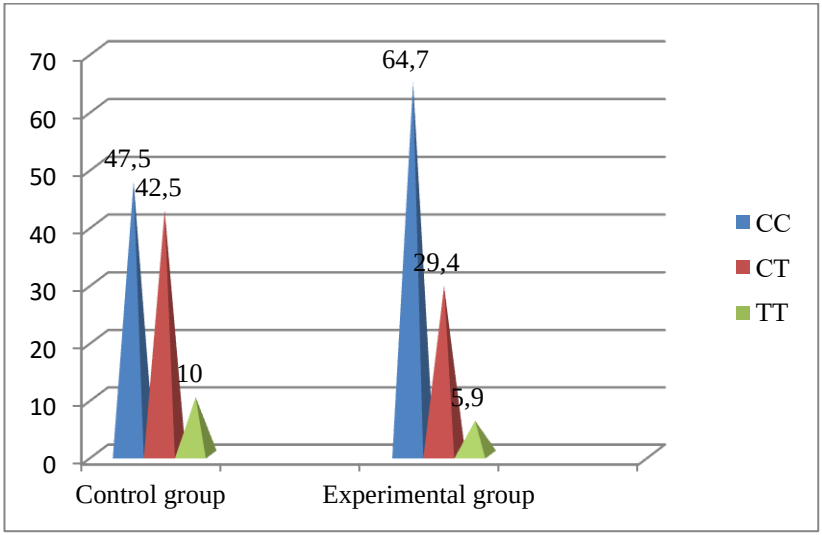
The distribution of polymorphic variants of genes MMP-2 (C^{-1306}→T), and TIMP-2 (G^{303}→A), in the studied groups

The studied gene		Control group n=80 (%)	Experimental group n=17 (%)
MMP2 (C^{-1306} →T)	**CC**	38 (47,5%)	11 (64,7%)
	CT	34 (42,5%)	5 (29,4%)
	TT	8 (10%)	1 (5,9%)
Hardy-Weinberg test (χ^2, p)		χ^2=0,01, p>0,05	χ^2=0,17, p>0,05
χ2 test, (χ^2, p)		-	χ^2=0,206, p>0,05

Analysis of the multiplicative model of inheritance of the MMP-2 gene (C^{-1306}→T), comparison of the control (n=80) and experimental groups with anastomotic leak (n=17) showed compliance with the distribution of genotypes according to Hardy-Weinberg's law (p>0,05), which was tested in the

control group using the test χ2 with 1 degree of freedom, without Yates correction. Using the test χ2 with 2 degrees of freedom, we did not find statistically significant differences in the distribution of genotypes in the group of sick people and the group of practically healthy people (p>0.05).

It is noteworthy that in the experimental group there were half as many carriers of the homozygous TT genotype as compared with the control group: 5,9% versus 10% (p>0,05), respectively. However, the number of carriers of the CC genotype dominant in all groups was greater in the group with anastomotic leak (experimental group): 64,7% versus 47,5% (p>0,05) in the control group (Picture 1).



Picture 1. Distribution frequency of allelic polymorphism (%) of the promoter (C^{-1306}→T) of MMP2 gene

Our data on the study of polymorphic variants of the MMP2 (C^{-1306} → T) genes in the Ukrainian population (n = 80) generally correspond to populations of Europe and the USA [5,6].

The closest genotypic variations in the studied genes were populations of Austria [7] and the Netherlands [8]. Moreover, we found significant differences when compared with the African and Asian populations [9]. Interestingly, in these populations, the frequency of the main C allele of the MMP-2 gene (rs243865) was 93.7% (Africa) and 90% (Asia), which significantly exceeds the indices of our control group (76%) and the European population (75,5 %). Whereas, the minor T allele was found in 24% of the control group, and 10% (Asia) and 6,7% (Africa), respectively [5].

As a result of genetic and statistical analysis of the polymorphism of the MMP-2 ($C^{-1306} \rightarrow T$) and TIMP-2 ($G^{303} \rightarrow A$) genes, variants of genotypes associated with the risk of development of anastomotic leak of the hollow digestive organs were determined.

Thus, in the experimental group with anastomotic leak, carriers of the homozygous SS genotype of the MMP2 gene were found to be 1,36 times more often than in the control group. At the same time, the minor TT homozygotes in the group of patients with anastomotic leak were almost half that in the control (5,9% versus 10% ($p < 0,05$)).

Conclusions.

1. Intestinal anastomotic leak is 1,36 times more common in carriers of homozygous CC genotype of the MMP-2 gene and twice less common in minor homozygotes of TT (5,9% vs. 10% ($p > 0,05$)).

2. Molecular genetic research can be a new promising area for the development of modern personalized diagnostic criteria and models for predicting the development and course of postoperative abdominal complications, including the anastomotic leak of the hollow digestive organs.

References:

1. Eto K, Urashima M, Kosuge M, et al. Standardization of surgical procedures to reduce risk of anastomotic leakage, reoperation, and surgical site infection in colorectal cancer surgery: a retrospective cohort study of 1189 patients. Int J Colorectal Dis 2018;33:755-62.

2. Pitel S, Lefèvre JH, Tiret E. Redo coloanal anastomosis: a retrospective study of 66 patients. Ann Surg. 2012; 256 (5):806-811.

3. Sharonne de Z, Usama AA, Rogier AR. Update of complications and functional outcome of the ileo-pouch anal anastomosis: overview of evidence and meta-analysis of 96 observational studies. Int. J. Colorectal Dis. 2012;27:843-853.

4. Marjanovic, G., Hopt, U. Physiologie der Anastomosenheilung. Chirurg 82, 41–47 (2011).

5. Population Diversity (Alleles in RefSNP orientation) / dbSNP Short Genetic Variation // NCBI. – 2017. – URL: https://www.ncbi.nlm.nih.gov/snp/rs243865/

6. Population Diversity (Alleles in RefSNP orientation) / dbSNP Short Genetic Variation // NCBI. – 2017. – URL: https://www.ncbi.nlm.nih.gov/snp/rs9900972

7. Mossbock G. Weger M. Faschinger C. Zimmermann C. Schmut O. Renner W. El-Shabrawi Y. Role of functional single nucleotide polymorphisms of MMP1, MMP2, and MMP9 in open angle glaucomas. Mol Vis. 2010;16).

8. van Diemen CC. Postma DS. Siedlinski M. Blokstra A. Smit HA. Boezen HM. Genetic variation in TIMP1 but not MMPs predict excess FEV1 decline in two general population-based cohorts.Respir Res. 2011;12:57.)

9. Xu E. Lai M. Lv B. Xing X. Huang Q. Xia X. A single nucleotide polymorphism in the matrix metalloproteinase-2 promoter is associated with colorectal cancer. Biochem Biophys Res Commun.2004;324:999–1003;

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THE ROLE OF OVEREXPRESSION OF CYCLOOXYGENASE-2 IN THE PROGNOSIS OF INVASIVE DUCTAL BREAST CANCER

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Introduction. The cyclooxygenases (COX) are enzymes involved in the metabolism of prostaglandins, thromboxane and prostacyclin. Under the influence of various factors (physical, chemical, inflammatory or mitogenic stimuli), arachidonic acid is released from the phospholipids of cell membranes under the action of phospholipase A2 [1]. Under the influence of COX, it is converted into an unstable intermediate prostaglandin H2 (PGH2), which under the action of tissue-specific isomerases is converted into numerous prostaglandins, thromboxane and prostacyclin. Prostaglandins activate specific receptors on cell membranes, belonging to the family of G-protein coupled receptors. Previously two species of the enzyme COX-1 and COX-2 were identified, which are encoded by different genes. Both COX genes are very similar, as are both isoenzymes, which have almost identical three-dimensional structures. The active positions of the two COX differ in only one amino acid residue. COX-1 is constitutively expressed in most tissues and organs, including the gastrointestinal tract, kidneys and platelets