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FEATURES OF HELICOBACTER PYLORI GENES IN NSAID GASTROPATHY IN PATIENTS WITH RHEUMATOID ARTHRITIS

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ABSTRACT

The article provides data on the result of a molecular genetic study of the genotypic characteristics of Helicobacter pylori in the formation of NSAIDs of gastropathy in patients with rheumatoid arthritis. Molecular-genetic study of the genotypic features of H. pylori in the formation of NSAID gastropathy in RA patients focuses on the prevention of gastroduodenal lesions.

The research is based on the genomic DNA of H. pylori, isolated from a biopsy of the antrum of 82 patients with RA and 22 healthy individuals. The genotype of H. pylori CagA, vacam1, vacam2, vacAs1, vacas2, vacas1b, vacas1c, icea1, icea2, was determined in biopsy samples.

Thus, according to the molecular genetic study, the pathogenic strain VacA m2 (x^2=4.12 p=0.011), IceA 2 (=6.71 p=0.036) prevails in patients with RA of the 2nd degree of activity.

Our preliminary results suggest that the H. Pylori VacA m2 and IceA 2 may be considered as additional markers of NSAID-gastropathy in rheumatoid arthritis.

Keywords: Helicobacter Pylori, rheumatoid arthritis, gastropathies, PCR (polymerase chain reaction), molecular genetic study, gastroduodenal diseases, cytotoxin.

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这篇文章提供了一项关于幽门螺杆菌基因型特征在类风湿性关节炎患者胃病非甾体抗炎药形成中的分子遗传学研究结果的数据。幽门螺杆菌基因型特征在 RA 患者中形成 NSAID 胃病的分子遗传学研究侧重于预防胃十二指肠病变。

该研究基于幽门螺杆菌的基因组 DNA，从 82 名 RA 患者和 22 名健康人的胃窦活检中分离出来。在活检样本中确定了幽门螺杆菌 CagA、vacam1、vacam2、vacAs1、vacas2、vacas1b、vacas1c、icea1、icea2 的基因型。

因此，根据分子遗传学研究，致病菌株 VacA m2 (x2=4.12 p=0.011)、IceA 2 (=6.71 p=0.036) 在 2 级活动度的 RA 患者中普遍存在。

我们的初步结果表明，H. Pylori VacA m2 和 IceA 2 可被视为类风湿性关节炎中 NSAID 胃病的额外标志物。

关键词：幽门螺杆菌，类风湿性关节炎，胃病，PCR（聚合酶链反应），分子遗传学研究，胃十二指肠疾病，细胞毒素。

INTRODUCTION

Rheumatoid arthritis (RA) – is progressive autoimmune disease of unknown etiology with predominant joint damage, characterized by the development of chronic erosive arthritis and frequent systemic inflammation of internal organs [1]. NSAIDs are significant component of the complex therapy on rheumatic diseases. At least 68.5% patients of rheumatoid arthritis constantly take NSAIDs [2]. Therefore, among patients with RA, there is a high frequency of NSAID-associated gastropathy, erosive and ulcerative lesions complicated by gastrointestinal bleeding and other complications [3]. Therefore, the use of non-steroidal antiinflammatory drugs (NSAIDs) is also a factor of ulcerogenesis. However, the influence of pathogenic factors of H. pylori on the likelihood of erosive and ulcerative damage to the gastroduodenal zone induced by NSAID intake has not been studied enough.

Numerous studies have focused on the prevalence and role of putative H. pylori virulence genes in the pathogenesis of diseases. The genomic sequence of H. pylori is very

diverse and is a powerful tool for understanding evolution of disease, to identify factors that cause a higher risk of severe consequences, and for finding new approaches to therapy [4]. Assessment of the pathogenicity of Helicobacter pylori is relatively complex, as H. pylori isolates demonstrate a high degree of geographic variation. At the same time, certain genotypes of H. pylori are associated with a more severe clinical outcome in some regions, while in other studied populations they are presented as practically harmless variants [7]. Moreover, the differences between East Asian and Western strains support the hypothesis that the degree of gastroduodenal pathology depends on the complex relationships between host genetics, environmental factors, and combinations of different H. pylori virulence genes [8]. Although the importance of most H. pylori virulence genes has not been uniformly explained yet, knowledge of their role in pathogenesis as well as disease outcome has improved noticeably over the past two decades. A number of studies have analyzed the relationship of Helicobacter pylori genes (cagA, vacAm1, vacAm2, vacAs1, vacAs1a, vacAs1b, vacAs1c, vacAs2, babA, iceA1, iceA2, dupA) with the development of gastritis, gastroduodenal ulcers and stomach cancer [9]. Research purpose: Molecular-genetic study of the genotypic features of H. pylori in the formation of NSAID gastropathy in RA patients for the prevention of gastroduodenal lesions.

MATERIALS AND METHODS

We analyzed 82 patients (71 (84%) women and 11 (16%) males) with rheumatoid arthritis in stationary treatment in the department of Rheumatology in TMA multidisciplinary clinic, who had been taking NSAIDs for a long time. The control group consisted of 22 healthy people. On the basis of laboratory examinations, the patients were divided into 2 groups according to the degree of activity of R.A. The material for the study was the genomic DNA of H. pylori isolated from a biopsy specimen of the antrum of the stomach. Molecular genetic studies were carried out at the Republican Scientific and Practical Medical Center of Hematology of the Republic of Uzbekistan. The molecular genetic part of the work included several stages: 1. Selection and optimization of the operation of oligoprimer systems for H. pylori virulence genes; 2. Collection of biological material; 3. Isolation of DNA from biological material; 4. PCR; 5. Electrophoresis and visualization of the results. The mutations was identified by the method Polymerase chain reaction (PCR) using popular primer sequences kindly provided by the Centre

of High Technologies. The PCR was performed using the selection of the firms Litech[a] (Russia) and Amplisens[a] (Russia). In all examined people, the genotype of H. pylori cagA, vacAm1, vacAm2, vacAs1, vacAs2, vacAs1b, vacAs1c, iceA1, iceA2 was determined in biopsy samples. The deviation of the distributions of genotypes of the studied DNA polymorphisms from the canonical Hardy-Weinberg distribution was assessed using the computer program for the analysis of genetic data “GenePop” (“Genetics of Population”) available on the Internet (http://wbiomed.curtin.edu.au/genepop).

The software package "OpenEpi 2009, Version 2.9" was used as a calculation tool.

RESULTS AND DISCUSSIONS

The human bacterial pathogen Helicobacter pylori has been the subject of intense research since its first description in 1984. And now it

affects half of the world's population [6]. The first sequence of the H. pylori genome was published in 1997 (Tomb et al. 1997), and it was the first bacterium for which two genome sequences became available in 1999. (Alm et al. 1999). These two strains showed high levels of genomic differences, both at the sequence level and at the predicted gene level. Helicobacter pylori lends itself to natural transformation, and mutations, recombination, and frequent genetic exchange have resulted in a high level of genome variability that can be observed over time even in one patient (Kuipers et al. 2000). The chromosome contains genes that encode a cluster of genes for urease, various cytotoxins and cag Pathogenicity island. Toxins include cytotoxin, a stretching cytotoxin, vacuolating cytotoxin (VacA), which induces apopoptosis of host epithelial cells (cell death), and a cytotoxin-associated antigen (CagA), which results in altered host cell signaling pathways. The CagA protein is translocated into host cells by a type IV secretion system encoded by pathogenicity islet cag[5].

Many studies have assessed the influence of genes of the microorganism H. pylori on the development of gastritis, peptic ulcer and stomach cancer, however, the information available in the literature on the role of the H. pylori genotype in the development of gastroduodenal diseases is contradictory.

As a result of the molecular genetic study, no statistically significant differences were found between the groups of patients in the degree of activity ($p > 0.05$). But at the same time, in the group of patients with the 2nd degree of RA activity, the spectrum of H. pylori genotypes was significantly different, vacAm2 and iceA2 were much more common. The vacA gene has 2 regions: signal - s (signal) and middle - m (middle). In the signaling s-region of the gene, two allelic variants are distinguished - s1 and s2. The middle m-region also has two allelic types - m1 or m2. The amount of VacA cytotoxin depends on the genotype of the strain. H. pylori strains vacA s1 m1 produce the greatest amount of VacA cytotoxin and are more often associated with peptic ulcers [5].

The genes cagA, vacAm1, vacAs1, vacAs1a, vacAs1b, vacAs1c, iceA1 were often identified in patients with I and II stage of RA activity. In patients who constantly took NSAIDs before therapy with Diclofenac sodium and continued to take them, did not lead to a change in the frequency of the spectrum of H. pylori genotypes. The results of molecular-genetic study were presented in the table 1.

Table 1

Indexes of molecular-genotypic study of H. pylori virulence genes of patients with RA of I and II degrees of activity

genotype H. pylori	**RA act I stage N= 33**		**RA act II stage N=49**		**Control group N=22**		**Statistics**
	N	%	N	%	N	%	
cagA	16	48	21	43	7	31	x^2=0,05 p=0.11
vacAm1	17	50	22	45	5	22	x^2=0,20 p=0.712
vacAm2	14	43	34	70	-	-	x^2=4,12 p=0.011
vacAs1	13	40	17	35	4	18	x^2=0,09 p=0.671
vacAs2	8	25	29	60	-	-	x^2=0,55 p=0.550
vacAs1b	2	6	3	7	1	5	x^2=0,09 p=1.09
iceA1	7	22	11	23	3	14	x^2=0,90 p=1.09
iceA2	10	30	25	52	-	-	x^2=6,71 p=0.036

Thus, according to the data of molecular-genetic study, the pathogenic strains VacA m2, IceA 2 prevail in patients with the second degree of RA activity.

CONCLUSION

This research is based on the genomic DNA of H. pylori, isolated from a biopsy of the antrum of patients with RA and healthy individuals. The results show that no statistically significant differences were found between the groups of patients in the degree of activity ($p > 0.05$), while, in the group of patients with the 2nd degree of RA activity, the spectrum of H. pylori genotypes was significantly different, vacAm2 and iceA2 were much more common. Our studies made it possible to select and optimize the operation of H. pylori gene oligoprimer systems. The developed methodology became the basis for genotyping of H. pylori genes in RA patients with and without gastropathy, which made it possible to carry out preliminary molecular genetic studies to determine the frequency of occurrence of allelic variants of the above genes among conditionally healthy and sick patients. Optimization of molecular genetic methods for detecting H. pylori virulence genes will help increase the efficiency and reduce the cost of the study. And our preliminary studies suggest that H. Pylori genes VacA m2, IceA 2 can be considered as additional markers of NSAID gastropathy in rheumatoid arthritis.

CONFLICT OF INTERESTS AND CONTRIBUTION OF AUTHORS

The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article and report on the contribution of each author.

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