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Features of the Occurrence of Combinations of H. Pylori Genotypes in Patients with Inflammatory-Ulceric Diseases of the Stomach

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Annotation: Target. To study the features of the occurrence of combinations of H. Pylori gene genotypes in patients with inflammatory and ulcerative diseases of the stomach in the Gastroenterology Clinic of the TMA.

Keywords: non-atrophic chronic gastritis, erosive chronic gastritis, gastric ulcer, risk of formation, Uzbekistan.

Introduction

Inflammatory and ulcerative lesions of the stomach are the most common pathologies among the entire group of stomach diseases [1,2]. These pathologies develop as a result of complex disorders in the gastric mucosa, arising from the interaction of agents of the external and internal environment [3].

Untimely diagnosis of inflammatory diseases of the stomach is very often fraught with the transition of inflammation in the gastric mucosa into chronic, ulcerative and even oncological processes [4]. In this regard, given the presence of many mechanisms of initiation of inflammatory and ulcerative diseases of the stomach that have not yet been fully revealed, modern researchers are particularly interested in studying them [5].

In recent years, more and more reports have appeared in the literature about a special contribution of *H. Pylori* gene genotypes in patients with inflammatory and ulcerative diseases of the stomach, which has a special contributionat the onset of complex disorders, ultimately leading to damage to the gastric mucosa [6, 7, 8, 9].

From numerous studies of the role of genotypic variants of *H. Pylori*, more than 30 genotypes of *H. Pylori* are known and the bacterium is found in the microflora of the gastric mucosa of patients and healthy people. They are combined into a region of the genome called the "pathogenicity island" (PAI).

According to our literature review, the demographic global prevalence of *H. pylori* infection is more than 50% [10].

*Cytotoxin-associated gene A (cagA). Helicobacter pylori and high-molecular protein CagA.*The cagA (cytotoxin-associated marker gene) gene is the most notorious virulence factor of *H. Pylori* and is recognized as the first bacterial oncogene. This protein is considered responsible for disruption of the integrity of the epithelium of the gastric mucosa, induction of uncontrolled proliferation of epithelial and lymphoid cells, secretion of proinflammatory cytokines, and the occurrence of an inflammatory reaction in the mucosa.

According to the data of Shiota S, Suzuki R, Yamaoka Y. (2013), *iceA* (induced by contact with epithelium) is activated upon contact with epithelial cells of the gastric mucosa (GM) and the gene exists in two allelic forms - *iceA1* and *iceA2* [11].

In patients infected with *H. Pylori* with the *iceA1* genotype, the infiltration of the lamina propria of the gastric mucosa by polymorphonuclear neutrophils is higher than in those infected with another genotype. Adhesion to gastric epithelial cells in vitro is induced by the expression of the *IceA1* protein. *H. Pylori iceA1* affects the immune response of gastric mucosa epithelial cells, promotes intensive production of proinflammatory cytokines (IL-6, IL-8, etc.) [11, 12].

Another factor of *H. Pylori* pathogenicity is the *vacA* gene (Vacuolating cytotoxin-associated gene) which codes for the synthesis of the vacuolating protein cytotoxin *VacA*, which causes vacuolization of gastric mucosa epithelial cells and their death. The vacA gene is present in the genome of all *H. Pylori* strains, has a mosaic structure and contains variable parts: the s-region (encodes the signal peptide) and the m-region (encodes the middle section of the protein). Allelic subtypes of this gene, different in size and nucleotide sequence, have been described: signal - *s1, s2*; middle - *m1* and *m2*, respectively [13].

The vacAs1m1 genotype has the highest level of cytotoxic activity and is associated with more severe diseases (peptic ulcer, gastric cancer), whereas the vacAs2m2 genotype of *H. Pylori* does not have significant cytotoxic potential [14, 15, 16].

It is known that there is a relationship between the vacA and cagA genotypes of *H. Pylori*. Most vacAsl strains are cagA-positive and have the highest activity for adhesion and intracellular infiltration. *H. Pylori* strains that produce active VacA protein (sl type) usually also contain cagA, and in strains that synthesize inactive VacA proteins (s2 type), the *cagA* gene is usually absent [17, 18, 19].

In substantiating the relevance of PUD, our Uzbek authors refer to the fact that the treatment of infection caused by *H. Pylori* is a difficult task, since there are no treatment regimens that guarantee 100% effectiveness. Our Uzbek researchers have found that Uzbekistan is one of the countries with a very high prevalence of *Helicobacter* infection: H. Pylori is diagnosed in 80% of the population. At the same time, 84% of the population of Uzbekistan have a mixed *iceA1-/iceA2*-genotype of *H. Pylori cagA*. In peptic ulcer disease, the pathogenic strain *cagA + vacA s1, vacA m2* and *iceA 1,2* prevails. In chronic gastritis type B associated with H. Pylori, the strain is *cag + vacA s1, vacA m2* and *iceA1*. The level of resistance of *H. Pylori* strains to clarithromycin reaches 13.3%. Prolongation of eradication therapy to 14 days and addition of bismuth tripotassium dicitrate (BTD) to it allows increasing the effectiveness of H. Pylori eradication to 95% [20].

A modern approach to studying *H.Ppylori* infection is to use molecular genetic methods of research. According to the literature, the polymerase chain reaction (PCR) method reveals the presence of the following pathogenicity factors: cytotoxin-associated genes: *cagA*, M, T, H, C, F, E; vacuolating cytotoxin A (*vacAs1* and *vacAs2*); the cytotoxicity gene iceA (induced by contact with epithelium); babA (blood-group-associated binding adhesion); hpaA – adhesin gene of Helicobacter pylori; oipA – outer inflammatory protein; alpB – adherence-associated lipoprotein B; the gene encoding the subunits of urease B (ureB) and I (ure I), etc.

The PCR method is characterized by high sensitivity and specificity, allows to detect both spiral and coccal forms of bacteria. Also, PCR testing allows to minimize false-positive results of *H. Pylori* isolation, to exclude the presence of other representatives of the genus *Helicobacter* in the biomaterial (for example, *Helicobacter suis* and *Helicobacter baculiformis*), whose role in the development of gastric pathology has not yet been fully established. Test systems of the company "DNA-technology" are often used. By agreement, it was decided to produce methods for diagnosing pathogenicity factors in the structure of the H. Pylori genome using the PCR method at the Republican Scientific and Practical Medical Center of Hematology of the Ministry of Health of the Republic of Uzbekistan.

In order to better understand the molecular genetic mechanisms of the formation of inflammatory and ulcerative processes in the gastric mucosa, conducting studies to assess the characteristics of the distribution of genotypes of *H. Pylori* genes in patients with inflammatory and ulcerative diseases of the stomach compared to healthy individuals living in Uzbekistan, as well as determining their relationship with an increased likelihood of developing these pathologies seems very interesting.

Material and methods. The study involved 96 patients (median age 55 years, standard deviation σ=51.6±17.1 years) with inflammatory and ulcerative lesions of the stomach, which amounted toa combined group of patients (Group 1, n=96) and 88 healthy individuals without inflammatory and ulcerative lesions of the stomach were formed (5th compared control group, n=88).

The 1st combined group of patients (n=96) was divided into the following groups: the 2nd group of patients with chronic non-atrophic gastritis (CNA, n=18); the 3rd group of patients with chronic erosive gastritis (CEG, n=23) and the 4th group of patients with gastric ulcer (GU, n=55).

All patients were observed at the clinic of the Tashkent Medical Academy (Tashkent, Uzbekistan) from 2019 to 2022.

Detection of genes genotypes of genesstrains *H. Pylori* by genes of virulence factors (*cagA, ice1, ice2* and *vacA gene with alleles m1, s2*) was carried out by the SNP-PCR method on a programmable thermal cycler of the company "Applied Biosystems" 2720 (USA), using test systems of the company "Litech" (Russia), according to the manufacturer's instructions. Isolation of *H. Pylori* DNA was carried out from gastric juice.

Mathematical processing of the results was carried out using the statistical software package “OpenEpi 2009, Version 9.3”.

Results.

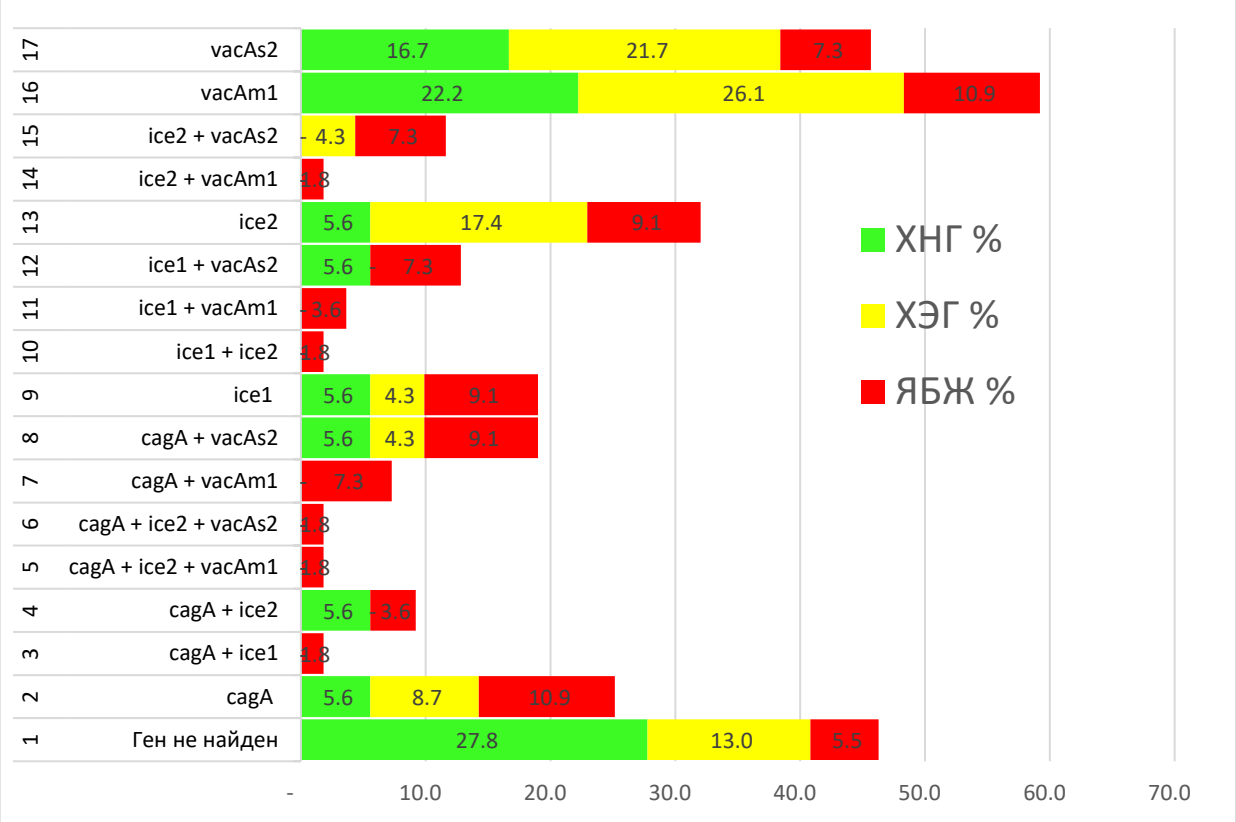
Results of the analysis of the distribution of frequencies of *H. Pylori* genotypes by virulence factor genes (*cagA, ice1, ice2* and *vacA* gene with alleles m1, s2) is given in table 1 or diagram 1

Table 1. Peculiarities of occurrence of combinations of genotypes of H. Pylori genes in patients with inflammatory and ulcerative diseases of the stomach

П/№	Генотипы HP	ХНГ		ХЭГ		ЯБЖ		Всего	
		n	%	n	%	n	%	n	%
1	Ген не найден	5	27,8	3	13,0	3	5,5	11	11,5
2	*cagA*	1	5,6	2	8,7	6	10,9	9	9,4
3	*cagA + ice1*	0	-	0	-	1	1,8	1	1,0
4	*cagA + ice2*	1	5,6	0	-	2	3,6	3	3,1
5	*cagA + ice2 + vacAm1*	0	-	0	-	1	1,8	1	1,0
6	*cagA + ice2 + vacAs2*	0	-	0	-	1	1,8	1	1,0
7	*cagA + vacAm1*	0	-	0	-	4	7,3	4	4,2
8	*cagA + vacAs2*	1	5,6	1	4,3	5	9,1	7	7,3
9	*ice1*	1	5,6	1	4,3	5	9,1	7	7,3
10	*ice1 + ice2*	0	-	0	-	1	1,8	1	1,0
11	*ice1 + vacAm1*	0	-	0	-	2	3,6	2	2,1
12	*ice1 + vacAs2*	1	5,6	0	-	4	7,3	5	5,2
13	*ice2*	1	5,6	4	17,4	5	9,1	10	10,4
14	*ice2 + vacAm1*	0	-	0	-	1	1,8	1	1,0
15	*ice2 + vacAs2*	0	-	1	4,3	4	7,3	5	5,2
16	*vacAm1*	4	22,2	6	26,1	6	10,9	16	16,7
17	*vacAs2*	3	16,7	5	21,7	4	7,3	12	12,5
	Всего	18	100	23	100	55	100	96	100

Frequency of genotype combinations									
P/No.	HP Genotypes	HNG		HEG		YABZ		Total	
		n	%	n	%	n	%	n	%
1	Gene not found	5	27.8	3	13.0	3	5.5	11	11.5
2	*cagA*	1	5.6	2	8.7	6	10.9	9	9.4
3	*cagA + ice1*	0	-	0	-	1	1.8	1	1.0

4	*cagA + ice2*	1	5.6	0	-	2	3.6	3	3.1
5	*cagA + ice2 + vacAm1*	0	-	0	-	1	1.8	1	1.0
6	*cagA + ice2 + vacAs2*	0	-	0	-	1	1.8	1	1.0
7	*cagA + vacAm1*	0	-	0	-	4	7.3	4	4.2
8	*cagA + vacAs2*	1	5.6	1	4.3	5	9.1	7	7.3
9	*ice1*	1	5.6	1	4.3	5	9.1	7	7.3
10	*ice1 + ice2*	0	-	0	-	1	1.8	1	1.0
11	*ice1 + vacAm1*	0	-	0	-	2	3.6	2	2.1
12	*ice1 + vacAs2*	1	5.6	0	-	4	7.3	5	5.2
13	*ice2*	1	5.6	4	17.4	5	9.1	10	10.4
14	*ice2 + vacAm1*	0	-	0	-	1	1.8	1	1.0
15	*ice2 + vacAs2*	0	-	1	4.3	4	7.3	5	5.2
16	*vacAm1*	4	22.2	6	26.1	6	10.9	16	16.7
17	*vacAs2*	3	16.7	5	21.7	4	7.3	12	12.5
	Total	18	100	23	100	55	100	96	100



Rice.1. Peculiarities of occurrence of combinations of genotypes of H. Pylori genes in patients with inflammatory and ulcerative diseases of the stomach

The results of a study to examine the prevalence of *H. Pylori* genotype variants in patients with inflammatory and ulcerative diseases of the stomach showed that, in total, among the examined patients (n = 96), bacteria were detected in 88.5% (n = 85) of patients.

Among all combinations of *H. Pylori* genotypes, cases with *cagA + vacAs2* were most frequently detected, accounting for 7.3% (n = 7), detected among patients with all forms of the disease: with CNG in 5.6% (n = 1), with CEG in 4.3% (n = 1), and with GU in 9.1% (n = 5) of cases.

The next combination of *H. Pylori* genotypes detected in 5.2% (n = 5) of cases was the *ice1 + vacAs2* variant, also detected among patients with all diseases: with CNG in 5.6% (n = 1), and with GU in 7.3% (n = 4) of cases.

In descending order, the combination *cagA + vacAm1* was further detected in 4.2% (n = 4) of patients, while among patients with CNG and CEG this combination was practically not detected, however, in 7.3% (n = 4) of cases it was detected in patients with GU.

The variant of the *ice2 + vacAs2* genotype combination, generally determined in 5.2% (n = 5) of patients, was most often detected in CEG and GU, respectively, accounting for 4.3% (n = 1) and 7.3% (n = 4) of cases, with this combination detected in 7.7% (n = 1) in patients with CEG.

In 3.1% (n = 3) of cases, a combination of the *cagA + ice2* genotype was detected, which was found only among patients with CNG 5.6% (n = 1) and GU 3.6% (n = 2).

In identical cases, which accounted for a total of 1.0% (n = 1), combinations of genotypes *ice2 + vacAm1* and *cagA + ice1* were identified, detected only in 1.8% (n = 1) of patients with GU, as well as combinations *ice1 + ice2*, *cagA + ice2 + vacAm1* and *cagA + ice2 + vacAs2*, detected only in 1.0% (n = 1) of patients with GU.

Analyzing the obtained results on the characteristics of the occurrence of combinations of *H. Pylori* genotypes, the following facts became obvious:

- in chronic hepatitis C, 4 combinations of genotypes were encountered: *cagA + vacAs2, ice1 + vacAs2, ice2 + vacAs2* and *cagA + ice2*.
- in HEG, 8 combinations of genotypes were encountered: *cagA + vacAs2, cagA + vacAm1, ice1 + vacAs2, ice2 + vacAs2, cagA + ice2, ice1 + vacAm1, ice2 + vacAm1* and *cagA + ice1*.
- in GU there were 7 combinations of genotypes: *cagA+ vacAs2; cagA+vacAm1; ice1+ vacAs2; ice2+ vacAs2; ice1+ ice2; cagA+ ice2+vacAm1* and *cagA+ ice2+ vacAs2*.

Thus, analyzing the above results, we have determined the features of the occurrence of combinations of *H. Pylori* genotypes at different degrees of severity of the course of inflammatory and ulcerative diseases of the stomach. Consequently, by determining the variant of combinations of pathogen genotypes, it is possible to determine the degree of virulence of *H. Pylori*, which is a very important early diagnostic criterion for predicting the clinical course and outcomes of inflammatory and ulcerative diseases of the stomach.

Conclusion. Inflammatory and ulcerative diseases of the stomach in terms of their pathogenetic aspects of onset to this day remain pathologies that have not been fully elucidated [2]. At the same time, given that the basis of this group of stomach diseases is an important place occupied by virulence factors (*cagA, ice1, ice2* and the *vacA* gene with alleles m1, s2) of *H. Pylori.*

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