ORIGINAL ARTICLE

RELATIONSHIP OF *HELICOBACTER PYLORI* CAGA AND VACA STATUS TO MORPHOLOGICAL CHANGES OF GASTRIC MUCOSA AND PRIMARY CLARITHROMYCIN RESISTANCE RATE IN PATIENTS WITH CHRONIC GASTRITIS: A CROSS-SECTIONAL STUDY

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ABSTRACT

The aim: To investigate the relation of *H. pylori* CagA and VacA status to morphological changes of gastric mucosa and primary clarithromycin resistance rate in patients with chronic gastritis.

Materials and methods: A cross-sectional study was conducted between May 2021 and January 2023, involving 64 patients with *H. pylori*-associated chronic gastritis. The patients were assigned to two groups according to the *H. pylori* virulence factors (CagA and VacA) status. The grades of inflammation, activity, atrophy, and metaplasia were determined according to the Houston-updated Sydney system. The identification of *H. pylori* genetic markers of antibiotic resistance and pathogenicity was performed by the polymerase chain reaction using paraffin stomach biopsies.

Results: Patients with CagA- and VacA-positive *H. pylori* strains had significantly higher grades of inflammation both in the antrum and in the corpus of the stomach, activity of gastritis in the antrum, higher incidence and grade of atrophy in the antrum. Primary resistance to clarithromycin was significantly more prevalent in patients with CagA- and VacA-negative *H. pylori* strains (58.3% vs. 11.5%, p=0.002).

Conclusions: Positive CagA and VacA status is related to more severe histopathological changes of gastric mucosa. In contrast, the rate of primary clarithromycin resistance is higher in patients CagA- and VacA-negative *H. pylori* strains.

KEY WORDS: *Helicobacter pylori*, CagA, VacA, gastritis, clarithromycin resistance

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INTRODUCTION

Gastric cancer (GC) is one of the leading causes of cancer mortality worldwide, with the highest incidence rates observed in the populations of Eastern Asia and Eastern Europe. According to the GLOBOCAN 2020 database, there were over 1 million new cases of GC and approximately 770,000 deaths attributed to it. If current trends persist, these figures are projected to double by 2040 [1].

One of the leading factors in the development of GC is *Helicobacter pylori* (*H. pylori*) infection, which induces a cascade of pathological changes in the gastric mucosa, including non-atrophic gastritis, atrophic gastritis, intestinal metaplasia, dysplasia, and cancer [2]. The risk of developing GC increases 18-fold with severe atrophic gastritis of the antrum, 90-fold with atrophy in the antrum and body, and 10-20-fold with intestinal metaplasia [3, 4]. However, it is worth noting that GC develops in only 1-2% of individuals infected with *H. pylori*, which is likely influenced by several factors, such as environmental factors, genetics, and the virulence of the *H. pylori* bacteria [2]. *H. pylori* virulence factors, such as those that express the cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) proteins, may lead to a more severe grade of inflammation and impact the development of atrophy and metaplasia in the gastric mucosa [5].

Effective eradication of *H. pylori* significantly reduces the risk of developing GC, but one of the most important barriers to this is antibiotic resistance [6]. This necessitates research into the relation between virulent strains of *H. pylori* and the clarithromycin resistance [7].

The effect of *H. pylori* virulence factors on the clinical, endoscopic, and morphological manifestations of *H. pylori*-induced gastritis also remains poorly understood.

THE AIM

To investigate the relation of *H. pylori* CagA and VacA status to morphological changes of gastric mucosa and primary clarithromycin resistance rate in patients with chronic gastritis.

MATERIALS AND METHODS

A cross-sectional study was conducted between May 2021 and January 2023, involving 64 patients over 18 years old with *H. pylori*-associated chronic gastritis (CG), who gave informed consent to participate. The patients were assigned to two groups according to the *H. pylori* virulence factors status. Group 1 included 38 patients with CagA- and VacA-positive *H. pylori* strains, and Group 2 included 26 patients with CagA- and VacA-negative *H. pylori* strains. The study was approved by the Ethical Committee of the Bogomolets National Medical University (protocol №159) and performed in accordance with the principles of the Helsinki Declaration.

The exclusion criteria included patients with gastric and duodenal ulcers, malignant tumors of the stomach, or other conditions that could significantly affect the study results, as well as individuals who had used antibiotics within 30 days or proton pump inhibitors within 2 weeks prior to the visit.

The average age of participants in Group 1 was 48.4±5.7 years, and in Group 2 it was 45.8±5.4 years. There were no statistically significant differences in age or gender between the two groups.

The diagnosis of CG was established based on esophagogastroduodenoscopy with proximal jejunoscopy, chromoscopy using an Olympus Evis Exera III device with high resolution and magnification up to ×115, chromoscopy, and narrow-band imaging (NBI), followed by morphological examination of stomach and duodenal biopsies. During the endoscopic examination, the condition of the gastric mucosa was evaluated, including the presence of edema, hyperemia, atrophy, and metaplasia. Biopsies were taken from all the affected areas diagnosed using high-resolution and high magnification endoscopy, chromoscopy, and NBI.

The biopsy specimens were processed using a carousel-type histoprocessor STP-120. An EC-350 station was used to fill the paraffin blocks, an HM-340E series rotary microtome was used to cut the paraffin blocks, and a Robot-Stainer HMS-740 automated staining system (all devices from Carl Zeiss MicroImaging GmbH, Hamburg, Germany) was used to stain the histological samples with hematoxylin-eosin and alcian blue. We used for examination an Axioskop 40 microscope with an Axio Cam MRc5 camera (Carl Zeiss).

The grades of inflammation, activity, atrophy, and metaplasia were determined according to the Houston-updated Sydney system.

The identification of *H. pylori* genetic markers of antibiotic resistance and pathogenicity was performed by the polymerase chain reaction (PCR) using paraffin stomach biopsies. A2142G/C and A2143G point mutations of the 23S rRNA gene were analyzed using a sequencing regime that allows analysis of short sequences, while T2182C point mutation was analyzed in a single nucleotide polymorphism search mode.

Detection of serum IgA, IgG antibodies to CagA and VacA was carried out by solid-phase enzyme-linked immunoabsorbent assay (ELISA). A SUNRISE photometric reader (Tecan, Austria), an Elx50 automatic washer (BioTeck, USA), and incubator-shakers were used.

Statistical analysis has been performed using Stata 11 and Statistica 6 software packages. The frequency characteristics in two independent groups were compared by the chi-square (χ^2) test. All statistical methods of analysis and calculated indicators were compared at a predetermined level of type I error (α) not exceeding 5% - $p<0.05$.

RESULTS

Studying the effect of *H. pylori* virulence factors on gastric mucosa inflammation, we found that patients with CagA- and VacA-positive *H. pylori* strains had a significantly higher grade of inflammation both in the antrum and in the corpus of the stomach (Table I).

The degree of CG activity was determined based on the severity of infiltration of the lamina propria and epithelial layer with neutrophilic leukocytes.

Table II demonstrates data on the degree of gastritis activity depending on the presence of *H. pylori* virulence factors. Thus, an active inflammatory process in the antrum of the stomach was found in 35 patients (54.7%), of whom 26 (68.4%) were in Group 1, and 9 (34.6%) were in Group 2. An active inflammatory process in the corpus was observed in 18 patients (28.1%), of whom 14 (36.8%) were in Group 1, and 4 (15.4%) were in Group 2. The degree of inflammatory activity in the antrum was statistically higher in patients with CagA- and VacA-positive *H. pylori* strains ($p<0.05$), while there was no significant difference between the two groups in the corpus of the stomach.

We also compared in both groups the cases of gastric mucosa atrophy in the antrum and corpus (Table III). Mu-

Table I. Grade of mucosal inflammation in the antrum and corpus of the stomach in patients with CagA- and VacA-positive and -negative strains of *H. pylori*

Inflammation	Group 1 (n = 38)	Group 2 (n = 26)	p_{1-2}	Group 1 (n = 38)	Group 2 (n = 26)	p_{1-2}
Localization	Antrum			Corpus		
Grade 1, n (%)	5 (13.1)	16 (61.5)	0.0001	12 (31.6)	26 (100)	0.0001
Grade 2, n (%)	27 (71.1)	10 (38.5)		6 (15.8)	0	
Grade 3, n (%)	6 (15.8)	0		20 (52.6)	0	

Table II. Degree of chronic gastritis activity in the antrum and corpus of the stomach in patients with CagA- and VacA-positive and -negative strains of *H. pylori*

Activity	Group 1 (n = 38)	Group 2 (n = 26)	p_{1-2}	Group 1 (n = 38)	Group 2 (n = 26)	p_{1-2}
Localization	Antrum			Corpus		
Present, n (%)	26 (68.4)	9 (34.6)	0.008	14 (36.8)	4 (15.4)	0.061
Absent, n (%)	12 (31.6)	17 (65.3)		24 (63.1)	22 (84.6)	
Degree 1, n (%)	8 (30.8)	6 (66.7)	0.048	14 (100)	4 (100)	1.00
Degree 2, n (%)	16 (61.5)	3 (33.3)		0	0	
Degree 3, n (%)	2 (7.7)	0		0	0	

Table III. Grade of atrophy in the antrum and corpus of the stomach in patients with CagA- and VacA-positive and -negative strains of *H. pylori*

Atrophy	Group 1 (n = 38)	Group 2 (n = 26)	p_{1-2}	Group 1 (n = 38)	Group 2 (n = 26)	p_{1-2}
Localization	Antrum			Corpus		
Present, n (%)	24 (63.1)	11 (42.3)	0.099	11 (28.9)	4 (15.4)	0.208
Absent, n (%)	14 (36.9)	15 (57.7)		27 (71.1)	22 (84.6)	
Grade 1, n (%)	11 (45.8)	10 (90.9)	0.012	11 (100)	4 (100)	1.00
Grade 2, n (%)	13 (54.2)	1 (11.1)		0	0	
Grade 3, n (%)	0	0		0	0	

Table IV. Presence of metaplasia and its type in the antrum and corpus of the stomach in patients with CagA- and VacA-positive and -negative strains of *H. pylori*

Metaplasia	Group 1 (n = 38)	Group 2 (n = 26)	p_{1-2}	Group 1 (n = 38)	Group 2 (n = 26)	p_{1-2}
Localization	Antrum			Corpus		
Present, n (%)	15 (39.5)	8 (30.8)	0.476	7 (18.4)	1 (3.8)	0.083
Absent, n (%)	23 (60.5)	18 (69.2)		31 (81.6)	25 (96.1)	
Type						
Complete (small intestinal), n (%)	11 (73.3)	6 (75)	0.931	4 (57.1)	1 (100)	0.408
Incomplete (colonic), n (%)	4 (26.6)	2 (25)		3 (42.9)	0	

Table V. Primary clarithromycin resistance rates in patients with CagA- and VacA-positive and -negative strains of *H. pylori*

Point mutations	Group 1A (n = 26)	Group 2A (n = 12)	p
T2182C, n (%)	2 (7.7)	5 (41.7)	0.022
A2143G, n (%)	1 (3.8)	2 (16.7)	0.229
A2142G/C, n (%)	0	0	-
Total	3 (11.5)	7 (58.3)	0.002

cosal atrophy in the antrum was detected in 24 (63.1%) patients in Group 1, and 11 (42.3%) patients in Group 2. Atrophy in the corpus was observed in 11 (28.9%) patients with *H. pylori* virulence factors, and 4 (15.4%) patients with CagA- and VacA-negative *H. pylori* strains. We found a statistically significant difference in the grade of atrophy

in the antrum between the groups. Patients with CagA- and VacA-positive *H. pylori* strains had a greater grade of atrophy (p=0.012). There was no statistically significant difference in the frequency and grade of atrophy in the stomach corpus between both groups.

Table IV shows data on the presence of intestinal metaplasia in the corpus and antrum of the stomach in both groups. In patients with *H. pylori* virulence factors, intestinal metaplasia in the antrum was observed in 15 (39.5%) individuals, with 11 cases of small intestinal metaplasia, and 4 cases of colonic metaplasia. In 8 (30.8%) patients in Group 2, metaplasia was found in the antrum, with 6 (75%) cases of small intestinal metaplasia and 2 (25%) cases of colonic metaplasia. We found no statistically significant difference in the incidence and type of metaplasia in the gastric antrum between the groups (p>0.05).

In the stomach corpus, intestinal metaplasia was detected in 7 (18.4%) patients in Group 1, and 1 (3.8%) patient in Group 2. Of the 7 patients in Group 1, 4 (57.1%) had small intestinal metaplasia and 3 (42.9%) had colonic metaplasia. 1 patient in Group 2 had small intestinal metaplasia. The observed occurrence of metaplasia in the stomach corpus did not exhibit a statistically significant difference between the two groups (p>0.05).

We studied the presence of primary resistance to clarithromycin in 38 patients, comprising 26 patients with CagA- and VacA-positive *H. pylori* strains (Group 1A), and 12 patients with CagA- and VacA-negative *H. pylori* strains (Group 2A). The identification of A2143G, A2142G/C, and T2182C point mutations in the V domain of 23S rRNA was used to determine the primary resistance to clarithromycin. 10 (26.3%) patients exhibited A2143G and T2182C point mutations out of the total number studied, as shown in Table V. Among the Group 1A patients, the point mutations were found in 3 patients (11.5%), whereas they were detected in 7 patients (58.3%) in another group. The results indicated that primary resistance to clarithromycin was significantly more prevalent in patients with CagA- and VacA-negative *H. pylori* strains (p=0.002).

DISCUSSION

Our findings suggest that patients with CG and *H. pylori* virulence factors (i.e., CagA and VacA) are statistically more likely to exhibit higher grades of inflammation in both the antrum and corpus of the stomach. Other researchers have noted that adhesion of *H. pylori* to gastric epithelial cells is associated with activation of several types of protein kinases (ERK, p38, JNK), which regulate the inflammatory process. JNK is predominantly activated during infection with CagA-positive *H. pylori* strains, which leads to a more pronounced inflammatory process [8]. The cascade of inflammatory responses is initiated as a result of pro-inflammatory cytokines production such as IL-8 and neutrophil activation factor by epithelial cells. The expression of these cytokines is particularly high in cases of infection with CagA-positive *H. pylori* strains, thereby suggesting a probable association between the presence of CagA- and VacA-positive *H. pylori* strains and heightened inflammation [9]. S. A. Boukhris et al. found a significant association between the presence of the CagA gene and increased neutrophil infiltration in patients under the age of 50 years old and stomach cancer in the age group over 50 years old. A relation has also been established between *H. pylori* VacA s1m1 genotypes, metaplasia, and gastric cancer [10]. Based on the results of our study, patients infected with CagA- and VacA-positive *H. pylori* strains exhibit a statistically significant increase in the level of gastritis activity in the antrum of the stomach, which is indicative of heightened neutrophil infiltration into the gastric lamina propria and the epithelial layer of the antrum. However, no statistically significant difference was observed between the groups with respect to inflammation in the corpus of the stomach. N. Almeida et al. found a significant correlation between CagA and the grade of metaplasia, neutrophil activity, and chronic inflammation. In addition, CagA- and VacA-positive *H. pylori* strains were associated with an increased risk of gastric ulcers [11]. N. Kim et al. observed a link between the incidence of gastric cancer and the existence of CagA and VacA m1 in patients over the age of 61 years old [12]. However, no association was detected between virulence factors and precancerous changes in the gastric mucosa in the study conducted by M. Akar et al. [13]. Our study revealed that patients with *H. pylori* virulence factors exhibit a significantly greater grade of atrophy in the antrum, while no statistically significant difference was observed in the corpus of the stomach. However, there was no statistically significant difference in the presence of metaplasia in the antrum and corpus of the stomach between the two groups. V. Shetty et al. noted that the combination of CagA- and VacA-positive *H. pylori* strains leads to peptic ulcer and gastric cancer [14]. Variations in several genes, besides CagA and VacA genotypes, may also be associated with the risk of inflammation and carcinogenesis, the data on which remain uncertain [15]. Thus far, there is no systemic analysis that has investigated the effect of bacterial virulence factors on the onset and progression of gastroduodenal diseases.

The elimination of *H. pylori* is deemed to be a cost-effective approach to mitigate the risk of GC. According to the Maastricht VI/Florence Consensus, the choice of treatment regimen should be based on the rate of the resistance of *H. pylori* strains to clarithromycin in a particular region [16]. Multicenter studies have shown that the primary level of clarithromycin resistance is more than 15% in majority of European countries, which makes

eradication regimens with clarithromycin ineffective [17]. There is a possible link between virulence factors and antimicrobial resistance. D.E. Brennan et al. found that patients with CagA-negative and VacA S2-positive strains had a high rate of primary resistance to clarithromycin (50.5%) [18]. Another cohort study found an increased frequency of A2143G point mutations among less virulent VacA i2 strains, and an increased frequency of A2142G point mutations in more virulent VacA i1 strains [19]. M. Karbalaei et al. showed the lower rate of resistance to antibiotics in patients with less virulent (VacA s2m2) strains [7]. Conversely, M. Bachir et al. did not find a link between virulence factors and clarithromycin resistance, which is consistent with the data from our study [20].

The limitations of a study are small sample size, inability to study other genes associated with the Cag Pathogenicity Island, and the lack of analysis pertaining to the various subtypes of VacA strains.

CONCLUSIONS

Our study reveals that patients with CagA- and VacA-positive *H. pylori* strains demonstrate significantly higher grades of inflammation in both the antrum and corpus of the stomach, activity of gastritis in the antrum, higher incidence and grade of atrophy in the antrum. We also noted that T2182C and A2143G mutations in the V domain of the 23S rRNA gene were more frequently detected in patients with CG and CagA- and VacA- negative strains of *H. pylori*.

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The Authors declare no conflict of interest.

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