Clinical manifestations and comorbid conditions in patients with chronic gastritis according to Helicobacter pylori CagA and VacA status

Objective — to determine the frequency of clinical manifestations and comorbidities in patients with Helicobacter pylori-associated chronic gastritis, depending on the presence of H. pylori cytotoxin-associated gene A and vacuolating cytotoxin A status.

Materials and methods. This cross-sectional study included 84 patients aged over 18 years with H. pylori-associated chronic gastritis (CG). Patients were divided into two groups: 50 patients with the cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) H. pylori strains and 34 patients with CagA– and VacA– H. pylori strains. H. pylori virulent strains (CagA+, VacA+) were detected using the polymerase chain reaction method. Solid-phase enzyme-linked immunosorbent assay (ELISA) was employed to detect immunoglobulins (IgA, IgG) against the CagA and VacA antigens of H. pylori. Analysis of medical records was conducted to assess clinical manifestations and the presence of comorbidities. Comorbid conditions were diagnosed using electrocardiography, abdominal ultrasound, and when necessary, computed tomography and magnetic resonance imaging, as well as videocolonoscopy. Statistical analysis was performed using the Stata 11 and Statistica 6 statistical software packages.

Results. Patients with CagA+ and VacA+ H. pylori strains were more likely to have epigastric pain (60 % vs. 35.3 %, p = 0.026) and constipation (52 % vs. 20.6 %, p = 0.004) than patients with CagA– and VacA– H. pylori strains. Among the associated comorbid conditions in patients with H. pylori-associated CG, the following were identified: gastroesophageal reflux disease — 33.3 %, irritable bowel syndrome — 35.7 %, non-alcoholic fatty liver disease — 23.8 %, autoimmune thyroiditis — 9.5 %, arterial hypertension — 10.7 %, and ischemic heart disease — 10.7 %. The irritable bowel syndrome was significantly more prevalent in patients with CagA+ and VacA+ H. pylori strains, compared to those without CagA and VacA strains (46 % vs. 20.6 %, p = 0.017). Other comorbid conditions occurred at similar frequencies among patients in both groups (p < 0.05).

Conclusions. Our study established that H. pylori virulent strains (CagA+, VacA+) could influence the clinical manifestations and associated conditions in patients with CG. Epigastric pain and constipation were more frequently observed in patients with H. pylori-associated CG, the following were identified: gastroesophageal reflux disease — 33.3 %, irritable bowel syndrome — 35.7 %, non-alcoholic fatty liver disease — 23.8 %, autoimmune thyroiditis — 9.5 %, arterial hypertension — 10.7 %, and ischemic heart disease — 10.7 %. The irritable bowel syndrome was significantly more prevalent in patients with CagA+ and VacA+ H. pylori strains, compared to those without CagA and VacA strains (46 % vs. 20.6 %, p = 0.017). Other comorbid conditions occurred at similar frequencies among patients in both groups (p < 0.05).

Keywords: Helicobacter pylori, virulence factors, chronic gastritis, irritable bowel syndrome, comorbidity.
Helicobacter pylori (H. pylori) infection affects in excess of 50% of the global populace and instigates the progression of pathological alterations within the gastric mucosa, thereby assuming a consequential role in the genesis of chronic gastritis (CG), peptic ulcer disease, gastric adenocarcinoma, and MALT lymphoma [10]. The correlation between H. pylori infection and gastroduodenal pathologies has been established, but the impact of the infection on the entire host organism remains debated. Increasingly, research points to H. pylori infection influencing the onset of numerous extragastric disorders as well as modifying their phenotype. An association between H. pylori and certain hematological, cardiovascular, dermatological, hepatobiliary, pancreatic, and neurodegenerative diseases, as well as colorectal cancer, has been elucidated [5]. The H. pylori virulence is contingent upon specific strains, their genotypic composition, and the correlated expression of virulence factors that facilitate the interaction between the infection and the host.

Certain investigations underscore the role of H. pylori virulence factors — CagA (cytotoxin-associated gene A) and VacA (vacuolating cytotoxin gene A), in the genesis of extragastric disorders, given their influence on systemic perturbations within the host organism. These ramifications encompass chronic immune system stimulation and extend to the establishment of molecular mimicry between bacterial and host proteins [10].

Objective — to determine the frequency of clinical manifestations and comorbidities in patients with Helicobacter pylori-associated chronic gastritis, depending on the presence or non-availability of H. pylori cytotoxin-associated gene A and vacuolating cytotoxin A status.

Materials and methods

This cross-sectional study was conducted from May 2021 to January 2023, involving 84 patients, aged over 18 years, with H. pylori-associated CG, who provided informed consent to participate. Patients were divided into two groups: 50 patients with the cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) H. pylori strains (Group 1) and 34 patients with CagA− and VacA− H. pylori strains (Group 2).

The study adhered to the Helsinki Declaration’s requirements and was approved by the Bioethics Expertise and Research Ethics Commission of the Bogomolets National Medical University (Protocol No. 159).

Exclusion criteria for the study encompassed patients diagnosed with gastric and duodenal ulcers, malignant tumors of the stomach, or other medical conditions that held the potential to exert substantial influence on the resultant outcomes of the investigation. Furthermore, individuals who had administered antibiotic regimens within the 30-day period antecedent to the study or proton pump inhibitors within a span of two weeks prior to their scheduled visit were not considered eligible for participation.

The diagnosis of CG was established through esophagogastroduodenoscopy with proximal jejunoscopy, using chromoendoscopy aided by the Olympus Evis Exera III system with high resolution, narrowband imaging, chromoendoscopy, and up to ×115 magnification for subsequent morphological examination of gastric and duodenal biopsy specimens.

The assessment of H. pylori virulence factors was executed utilizing the polymerase chain reaction (PCR) technique, employing paraffin-embedded gastric biopsy specimens as the biological source. DNA extraction from the biopsy samples was achieved by utilizing the QIAamp DNA FFPE Tissue Kit (QIAGEN). Subsequent to the DNA isolation procedure, targeted amplification of specific segments within the CagA and VacA genes was undertaken. The PCR amplification was performed employing the CFX thermal cycler (BioRad).

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.

In addition to endoscopic and histological examinations, patients’ complaints and their dynamics throughout the treatment process were analyzed. Detailed medical history, including the course of the disease and personal history, as well as family medical history, was examined. Objective assessments were performed through clinical examinations, and medical documentation was thoroughly reviewed. For all participants in the study, an analysis of medical records was carried out to evaluate the presence of concomitant disease. For diagnosing concomitant conditions, electrocardiography, ultrasonographic examination of abdominal organs, and when necessary, computed tomography and magnetic resonance imaging were employed. Video colonoscopy was also utilized as part of the diagnostic process.

Stata 11 and Statistica 6 software packages were used for statistical analysis. The normality of data distribution was assessed with the Shapiro-Wilk test. Continuous data were presented as mean and standard deviation (M ± SD), proportions were reported as number of cases and frequency. Mann-Whitney U test was used to compare the means.
of 2 non-normal, continuous data. The frequency characteristics in two independent groups were compared by the chi-square ($\chi^2$) test. In cases with a small number of patients in subgroups of certain clinical parameters (5 or fewer), the significance of between-group differences in frequency characteristics was evaluated using Fisher’s exact test. A p-value < 0.05 was considered statistically significant.

**Results**

Clinical and anamnestic characteristics of the patients are presented in Table 1. The examined groups did not differ in terms of age, gender distribution, body mass index, and mean duration of symptoms.

During the clinical examination of patients in both groups, diverse clinical manifestations were identified. Table 2 demonstrates data on the frequency of clinical symptoms in patients with CG depending on the *H. pylori* virulence factors status. Patients with CagA+ and VacA+ *H. pylori* strains were more likely to have epigastric pain and constipation than patients with CagA− and VacA− *H. pylori* strains (60% vs. 35.3%, p = 0.026 and 52% vs. 20.6%, p = 0.004). No statistical difference was observed between other symptoms in two groups (p > 0.05).

Comorbidities were identified in both groups of patients. Overall, among patients with *H. pylori*-associated CG, the following conditions were observed: gastroesophageal reflux disease (GERD) in 33.3%, irritable bowel syndrome (IBS) in 35.7%, non-alcoholic fatty liver disease (NAFLD) in 23.8%, autoimmune thyroiditis (AIT) in 9.5%, arterial hypertension (AH) in 10.7%, and ischemic heart disease (IHD) in 10.7%. Table 3 shows data on the frequency of comorbid conditions in patients with CG depending on the *H. pylori* virulence factors status.

Patients with CagA+ and VacA+ *H. pylori* strains significantly more often exhibited irritable bowel syndrome (46% compared to 20.6%, p = 0.017). Comorbidities such as GERD, NAFLD, AIT, AH, and IHD were detected in patients of both groups with comparable frequencies (p < 0.05).

**Discussion**

Our study revealed that the majority of patients with *H. pylori*-associated CG and CagA+ and VacA+ status was statistically more likely to have...
complaints of epigastric pain and constipation, and among comorbidities, irritable bowel syndrome was prevalent. C. Wang et al. supports the notion that *H. pylori* infection is associated with an increased risk of IBS, and its eradication improves clinical manifestations [12]. Z. Wang et al. demonstrated that patients with IBS more often had *H. pylori* CagA+ status [13]. Other researchers have also found that patients with *H. pylori* infection have an increased risk of IBS, and bacterial eradication may reduce the risk of developing this syndrome [4, 6]. Through its virulence factors, *H. pylori* increases the activation of mast cells and immune activity in the gastrointestinal tract (GIT), which may correlate with symptoms of visceral hypersensitivity [1]. J. Yakoob et al. demonstrated that patients with IBS and diarrhea more frequently exhibit *H. pylori* infection with virulent CagA+ strains [14]. Conversely, Q. X. Ng et al. meta-analysis contradicted the association between *H. pylori* and irritable bowel syndrome [8]. This issue requires further meticulous research with larger sample sizes and assessments following *H. pylori* eradication therapy. A lot of studies suggest that *H. pylori* strains containing the CagA protein are associated with extragastric conditions such as atherosclerosis, metabolic syndrome, and Behçet’s disease, but it remains uncertain whether the CagA protein can impact the lower gastrointestinal tract [10]. Some researchers have demonstrated that *H. pylori* infection can initiate inflammation in the lower parts of the gastrointestinal tract and play a role in the development of colorectal cancer. However, at the same time, it possesses protective properties for patients with inflammatory bowel diseases [11]. H. Zhang conducted experimental research on mice with colitis and found that *H. pylori* gastric colonization reduces intestinal inflammation and influences the immune homeostasis of the colon [16]. In recent years, several comprehensive reviews have been published, which prove that *H. pylori* infection affects the occurrence of numerous dermatological, neurological, ophthalmological, hematological, cardiovascular, allergic, metabolic, and hepatobiliary diseases. Most of these diseases have multifactorial pathogenesis, and *H. pylori* infection may play a role in their development. Indeed, meta-analyses have revealed that *H. pylori* infection increases the risk of NAFLD in middle-aged patients [7]. S. Yang et al. demonstrated a direct association between atherosclerosis and *H. pylori* infection, and they further hypothesized that prevention and eradication of *H. pylori* CagA+ strains could reduce the frequency of atherosclerosis and its related events [15]. N. Figura et al. demonstrated that *H. pylori* infection with CagA+ strains is linked to an increased risk of thyrotoxicosis and autoimmune thyroiditis, likely through the activation of systemic inflammation and molecular mimicry [3]. The role of *H. pylori* infection in the development of GERD remains controversial [9]. T. Zhao et al. concluded that *H. pylori* eradication reduces esophageal peristalsis, increases exposure to esophageal acid, and exacerbates GERD symptoms, suggesting a potentially protective effect of *H. pylori* infection for GERD patients [17]. A meta-analysis by Y. Fang et al. confirmed that *H. pylori* is a significant risk factor for hypertension. Patients infected with *H. pylori* had a 13.4% higher risk of hypertension compared to non-infected individuals [2]. In our study, we did not find a significant difference between the mentioned comorbidities and the presence of *H. pylori* virulence factors (CagA+, VacA+). There has been a growing body of research focusing on the extragastric effects of *H. pylori* and its virulence factors. However, many studies are limited by sample size, unclear understanding of the underlying mechanisms, and constraints in *H. pylori* detection methods. Therefore, further investigations are necessary to better understand the role of *H. pylori* infection and its strains in developing new preventive and therapeutic approaches. Our study has been limited by the small sample size, impossibility to examine other genes of the Cag virulence island, and insufficient research of the eradication therapy effects on the associated conditions progression. Also, the patients’ socioeconomic status was not regarded.

**Conclusions**

*H. pylori* CagA+ and VacA+ status is an important factor that may affect the clinical manifestation and comorbidities of *H. pylori*-associated CG. Patients with *H. pylori* CagA+ and VacA+ status exhibit a higher prevalence of clinical symptoms, particularly epigastric pain and constipation, compared to patients without virulent strains (CagA–, VacA–). The irritable bowel syndrome was significantly more common in patients with CagA+ and VacA+ *H. pylori* strains.

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References


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Клінічні вияви та коморбідні стани у пацієнтів із хронічним Helicobacter pylori-асоційованим гастритом та високотоксигенними штамами CagA та VacA

Мета — визначити частоту клінічних виявів та супутніх захворювань у пацієнтів із хронічним Helicobacter pylori-асоційованим гастритом залежно від наявності чи відсутності високотоксигенних штамів H. pylori (цитотоксин А-асоційований (CagA) та вакуолізувальний цитотоксин А-асоційований (VacA)).

Матеріали та методи. До корп-секційного дослідження було залучено 84 пацієнтів віком понад 18 років із хронічним гастритом (ХГ), асоційованим з H. pylori. Пацієнтів розподілили на дві групи: 50 осіб із високотоксигенними штамами H. pylori (CagA+, VacA+) та 34 без високотоксигенних штамів. Високотоксигенні штани H. pylori (CagA+, VacA+) виявили методом полімеразної ланцюгової реакції, імуноглобули (Iga, IgG) до антигенів білків CagA і VacA бактерії H. pylori — за допомогою керрфракці-
го імуноферментного аналізу. Проводили аналіз медичної документації для оцінки клінічних виявів та визначення супутньої патології. Для діагностики супутніх захворювань використовували електрокардіографію, ультразвукове дослідження органів черевної порожнини, за потреби — комп'ютерну та магнітно-резонансну томографію, відеоколоноскопію. Статистичну обробку проводили з використанням пакетів статистичних програм Stata 11 та Statistica 6.

Результати. У пацієнтів зі штамами H. pylori CagA і VacA частіше реєстрували скарги на епігастральний біль (у 60,0 та 35,3 %, \( p = 0,026 \)) та запори (у 52,0 і 20,6 %, \( p = 0,004 \)). Із супутніх захворювань у пацієнтів із H. pylori-асоційованим ХГ виявлено гастроезофагеальну рефлюксну хворобу (33,3 %), синдром подразненого кишечника (35,7 %), неалкогольну жирову хворобу печінки (23,8 %), артеріальну гіпертензію (9,5 %), ішемічну хворобу серця (10,7 %). Синдром подразненого кишечника статистично значущо часто виявляли в пацієнтів зі штамами H. pylori CagA та VacA порівняно з особами без цих штамів (у 46,0 і 20,6 %, \( p = 0,017 \)). Інші коморбідні стани траплялися з однаковою часто- тою у пацієнтів обох груп (\( p < 0,05 \)).

Висновки. Установлено, що високотоксигенні штами H. pylori (CagA-, VacA-), можуть впливати на клінічні вияви та супутні захворювання у пацієнтів з ХГ. Біль в епігастрії та запори частіше реєстрували в пацієнтів зі штамами H. pylori CagA і VacA, а із супутніх захворювань у них частіше виявляли синдром подразненого кишечника. Ерадикаційна терапія може бути методом профілактики цієї коморбідності, але для підтвердження гіпотези слід провести додаткові дослідження.

Ключові слова: Helicobacter pylori, фактори вірулентності, хронічний гастрит, синдром подразненого кишечника, коморбідність.

ДЛЯ ЦИТУВАННЯ