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STUDY ON THE *oipA, babA2, cagE* AND *cagA* GENES OF *HELICOBACTER PYLORI* IN PATIENTS WITH GASTRITIS, PEPTIC ULCER DISEASE

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Thesis could be found in:

1. National Library of Vietnam
2. Hue's university learning resource center
3. Library of University of Medicine and Pharmacy, Hue University

INTRODUCTION

1. Rationale for the study

The *Helicobacter pylori (H. pylori)* bacterium is the leading cause of chronic gastritis, peptic ulcers, and gastric cancer. Although the prevalence of *H. pylori* infection is relatively high (48.5%), in fact, only 10-20% of patients infected with *H. pylori* progress to peptic ulcers and 1-2% progress to gastric cancer. This could be explained partly by the differences in the virulence characteristics of *H. pylori* strains. Currently, in addition to the role of the *cagA* gene encoding the classic virulence factor *CagA*, the role of *cag* genes encoding proteins of the type IV secretion system (T4SS) and genes encoding outer membrane proteins of *H. pylori* has been mentioned. The *cagE* gene encodes the *CagE* protein of T4SS. The *babA2* gene encodes the BabA protein, the first identified adhesin of *H. pylori*. The *oipA* gene encodes the protein OipA, which has the functional status "on/off" depending on the number of CT repeats in the 5' region, regulated by the slipped strand mispairing mechanism. In Vietnam, there is no study on the four genes of the *oipA, babA2, cagE, and cagA* of *H. pylori*. Therefore, we conducted the study "Study on the *oipA, babA2, cagE, and cagA* genes of *Helicobacter pylori* in patients with gastritis, and peptic ulcer disease."

2. Research objectives

1. To determine the prevalence of the genes and the gene combinations of *oipA* "on/off", *babA2*, *cagE*, and *cagA* of *Helicobacter pylori* in patients with chronic gastritis and peptic ulcer disease.

2. To investigate the association between each gene and the gene combinations of *oipA* "on/off," *babA2, cagE, and cagA* of *Helicobacter pylori* and chronic gastritis and peptic ulcer disease.

3. Scientific and practical significance

The study provides the prevalence of *oipA "on/off", babA2, cagE,* and *cagA* genes of *H. pylori* strains and the association between them and gastritis, and peptic ulcer disease. The results provide information about *H. pylori* strains carrying highly virulent genes associated with an increased risk of peptic ulcers and chronic gastritis with precancerous lesions. This is one of the keys to the selective eradication of *H. pylori* to prevent the progression of severe gastroduodenal diseases.

4. New contributions of the thesis

The study notes that the prevalence of H. *pylori* strains carrying the *oipA* "on" gene is 96.0%, *babA2* (+) is 74.6%, *cagE* (+) is 83.8%, and *cagA* (+) is 83.8%, contributing to a better understanding of the molecular characteristics of this bacterium in Vietnam.

The study provides information on *H. pylori* strains carrying high-virulence genes and gene combinations, which are associated with an increased risk of peptic ulcers and chronic gastritis with precancerous lesions, elucidating the role of these genes in the development of *H. pylori*-induced gastritis and peptic ulcer disease.

5. Thesis layout

The thesis consists of 117 pages: 3 pages of introduction, 29 pages of literature overview, 24 pages of material and methods, 27 pages of study results, 31 pages of discussion, 2 pages of conclusion, and 1 page of recommendations. The thesis has 31 tables, 12 figures,

7 charts, and 141 references (20 documents in Vietnamese and 121 documents in English).

Chapter 1: LITERATURE OVERVIEW

1.1. OVERVIEW OF *HELICOBACTER PYLORI*

1.1.1. Epidemiology

The worldwide prevalence of *H. pylori* infection is 48.5%; in Vietnam, it ranges from 55.4% to 63.7%. Risk factors for *H. pylori* infection include low socioeconomic conditions. The transmission routes of *H. pylori* include oral-oral, fecal-oral, and gastric-oral routes.

1.1.2. The microbiological characteristics of H. pylori and methods for diagnosing Helicobacter pylori infection

1.1.2.1. The microbiological characteristics of H. pylori

H. pylori is a Gram-negative bacterium, ranging from 2-4 µm in length, curved or spiral-shaped, with 2-6 flagella at one end. *H. pylori* is a microaerophilic bacterium that grows slowly and requires specialized culture conditions in the laboratory.

1.1.2.2. Methods for diagnosing Helicobacter pylori infection

* *Invasive methods*: histology, culture, rapid urease test.

**Non-invasive methods:* ure breath test, stool antigen test, serology.

* *Molecular biology method:* PCR: Polymerase chain reaction.

1.1.3. The pathogenic mechanism of H. pylori

Four steps for colonization and pathogenesis of *H.pylori*: (1) Survival under acidic stomach conditions by urease (2) movement toward epithelium cells via flagella (3) binding to host receptors by adhesins; (4) causing tissue damage by toxin release.

1.1.4. The H. pylori-induced chronic gastritis and peptic ulcer disease

1.1.4.1. Chronic gastritis and precancerous lesions

Definition of chronic gastritis

Chronic gastritis (CG) is characterized by chronic damage of the gastric mucosal epithelium, which can lead to significant histopathological changes such as atrophy, intestinal metaplasia, and dysplasia, upon which gastric cancer may develop.

The H. pylori-induced gastric precancerous lesions

The process of developing intestinal-type gastric cancer due to *H. pylori* infection, as described by Correa, progresses through a precancerous cascade: atrophy → intestinal metaplasia → dysplasia, and finally gastric carcinoma.

Gastric atrophy is a precancerous lesion characterized by histological changes with the presence of chronic inflammatory cells including lymphocytes and plasma cells in the lamina propria and loss of gastric glands.

Intestinal metaplasia represents a phenotypic change from the normal epithelial cell of the gastric mucosa to an intestinal phenotype.

Dysplasia is characterized by abnormalities in the morphology and structure of glands and glandular epithelial cells in the gastric mucosa. Histological changes include cytological atypia, glandular architectural distortion, and cytoplasmic differentiation.

1.1.4.2. The H. pylori-induced peptic ulcers

Peptic ulcers (PU) are localized defects of the gastroduodenal mucosa, extending at least to the depth of the muscularis mucosa

with a minimum diameter of 5mm, diagnosed through endoscopy. *H. pylori* infection is associated with approximately 70% of gastric ulcers and 80% of duodenal ulcers.

1.2. THE GENES of *oipA "on/off", babA2, cagE,* AND *cagA* OF *HELICOBACTER PYLORI*

1.2.2. The oipA gene and OipA protein

The outer inflammatory protein A (*OipA*) is a pro-inflammatory and adhesin of *H. pylori*. The *oipA* gene encodes the outer membrane protein *OipA*, which has an "on/off" status depending on the number of CT (C (cytosine), T (thymine)) repeats in the 5' region of the gene, regulated by the slipped strand mispairing mechanism. The pattern of CT repeats is geographically specific. The *oipA* “on” gene encodes the functional *OipA* protein, serves as an adhesin, and increases interleukin- 8 (IL-8) production. The *oipA* “on” gene has been shown by several studies associated with an increased risk for peptic ulcers (PU) and gastric cancer.

1.2.2. The babA2 gene and BabA Protein

The BabA protein (BabA: blood group antigen binding adhesin) with a molecular weight of 75-80 kDa, is the first identified adhesin of *H. pylori*. The *babA2* gene encodes the BabA protein. The *babA2* gene is shown to be associated with an increased risk of peptic ulcers (PU) in Western countries; however, this association has not been identified in East Asian countries. Additionally, some studies have reported that the *babA2* gene is associated with an increased risk of intestinal metaplasia and gastric cancer.

1.2.3 The cagE gene and CagE Protein

The *CagE* protein is a component of the type IV secretion system (T4SS) of the Cag pathogenicity island (CagPAI). The *cagE* gene encodes the *CagE* protein. The *cagE* gene is often considered a gene marker of the presence of *cag*PAI, which has been reported to be associated with severe gastroduodenal diseases.

1.2.4. The cagA gene and CagA Protein

The *CagA* protein is the main virulence factor of *H. pylori*, encoded by the *cagA* gene located at the 3' end of the *cag* pathogenicity island (cagPAI). The *cagA* gene has been reported to be associated with peptic ulcers (PU) and gastric cancer (GC).

1.3. THE INTERNATIONAL AND DOMESTIC STUDIES

1.3.1. The international studies

In 2012, Sahara found that the *cagA*(+) gene was related to an increased risk of PU (OR= 2.83, 95%CI: 1.50 - 5.34, p = 0.001).

In 2013, Liu observed a significant association between the *oipA* "on" gene and an increased risk of PU (OR = 3.97, 95%CI: 2.89-5.45) and GC (OR = 2.43, 95%CI: 1.45-4.07).

In 2017, Khatoon found that the prevalence of *cagA(+)* and *cagE(+)* genes was 73% and 83%, respectively. The *cagE(+)* gene increased the risk of PU by 5 times (95%CI: 2.31–8.22) and GC by 3 times (95%CI: 1.82–6.13).

In 2020, Bartpho identified that the *babA2(+)* gene was not associated with an increased risk of precancerous gastric lesions.

1.3.2. The domestic studies

In 2010, Nguyen Lam Tung studied in Hanoi and Ho Chi Minh City and found that the prevalence of the *cagA*(+) gene, *cagE*(+) gene, and *oipA* "on" gene was 95%, 88%, and 100%, respectively.

In 2021, Nguyen Thi Mai Ngan conducted a study in Hue and found that the prevalence of the *cagA*(+) gene in patients with gastritis and peptic ulcer disease was 77.9%. There was no association between the *cagA* gene and gastroduodenal diseases.

Chapter 2: STUDY OBJECTS AND METHODS

2.1. STUDY OBJECTS

Patients with gastritis and peptic ulcer disease infected with *H. pylori* met the inclusion and exclusion criteria from May 2021 to October 2022 at Can Tho University of Medicine and Pharmacy Hospital.

2.1.1. Inclusion criteria

- Patients with clinical symptoms of gastroduodenal diseases such as epigastric pain, burning, nausea, vomiting, regurgitation, sour belching, bloating, indigestion, and loss of appetite underwent upper gastrointestinal endoscopy and gastric sample biopsy.
- Patients diagnosed with chronic gastritis, peptic ulcer disease:
    - Chronic gastritis is confirmed by inflammatory lesions on endoscopic images and chronic inflammation in histopathological assessment according to the updated Sydney system.
    - Peptic ulcer disease is confirmed by ulcerative lesions in the stomach and/or duodenum on endoscopic images.
- Patients had a positive diagnosis for *H. pylori* infection in both rapid urease test and culture methods.

2.1.2. Exclusion criteria

- Patients consumed Histamine H2 receptor antagonists or proton pump inhibitors for the preceding two weeks and antibiotics or bismuth for four weeks before endoscopy.
- Patients had a history of upper gastrointestinal surgery or bleeding disorder.

- DNA extracted from isolated *H. pylori* strains was insufficient for the quality and quantity requirements for molecular biological testing.

- Patients have bad sequencing results of the *oipA* gene or incompatible PCR results for *cagA, cagE,* and cagPAI-empty.

2.2. STUDY METHODS

2.2.1. Study design: a cross-sectional study.

2.2.2. Sample size: The minimum required sample size was calculated using the formula:

$$n \geq \frac{Z^2_{1-\alpha/2} \times p(1-p)}{d^2}$$

with Z (1- α/2) =1.96 with α = 0.05, absolute error d = 0.08, p_1=0.779, p_2=0.5 the calculated sample size ≥ 151. The actual sample size was 173 patients.

2.2.3. Research procedure

- Patients referred for upper gastrointestinal endoscopy at the Centre of Endoscopy - Interventional Endoscopy, Can Tho University of Medicine and Pharmacy Hospital, were counseled and voluntarily consented to participate in the study.

- An upper gastrointestinal endoscopy was performed to record gastritis or peptic ulcers, and two gastric biopsy samples were taken for a rapid urease test and *H. pylori* culture. Patients with endoscopic gastritis had two additional gastric biopsies for histopathological examination.

- Patients with positive rapid urease test results continued with culture *H. pylori* in the Department of Microbiology, Can Tho University of Medicine and Pharmacy. *H. pylori* was identified using Gram staining and urease, catalase, and oxidase tests.

- Isolated *H. pylori* strains were stored in TE Buffer solution at -20°C and then transferred to the Molecular Genetics Laboratory - Department of Medical Genetics, University of Medicine and Pharmacy, Hue University for DNA extraction and then, PCR performed using specific primers for *H. pylori (ureC)*, and *oipA, babA2, cagE,* and *cagA* genes.

- The *oipA* gene "on/off" status was determined using the Sanger sequencing of the region containing the CT repeats.

2.2.4. Study variables

Study variables were defined and listed in detail.

2.2.5. The procedures and techniques used in the study

The procedures and techniques used were described in detail.

2.2.6. Data analysis

The data were analyzed using SPSS 26.0 and R Statistical Environment v4.2.2. Quantitative variables were described using means with standard deviations, while categorical variables were described using frequencies and percentages. The chi-square test or Fisher's exact test was used to compare proportions' differences. Odds ratios (OR) and their 95% confidence intervals (95% CI) were calculated in univariate binary logistic regression analysis to investigate the association between genes or gene combinations and chronic gastritis and peptic ulcer disease. Firth logistic regression analysis was used when the distribution of chronic gastritis and peptic ulcer disease according to the studied genes had frequencies as 0. Statistical significance was considered when $p < 0.05$.

2.2.7. Study Ethics

The study was approved by the Biomedical Research Ethics Committee, Hue University of Medicine and Pharmacy, Hue University, with code H2021/389.

Chapter 3: RESULTS

3.1. General Characteristics of the Study Sample

3.*1.1. Age, Gender, Smoking*

The mean age of the studied patients was 42.60 ± 15.10 years. The age group ≥40 accounted for the majority (53.2%). Males comprised 49.1%. Patients with smoking accounted for 20.2%.

3.1.2. The characteristics of chronic gastritis and peptic ulcer disease

3.1.2.1. The distribution of chronic gastritis and peptic ulcer disease

In 173 studied patients, chronic gastritis with precancerous lesions accounted for 53.7%; peptic ulcers accounted for 24.3%; chronic gastritis without precancerous lesions accounted for 22.0%.

3.1.2.2. The association between age group and gender and chronic gastritis and peptic ulcer disease

There was a statistically significant difference in the distribution of age groups and gender among patients with chronic gastritis and peptic ulcer disease ($p<0.05$).

3.1.3. The clinical symtoms

The most common clinical symptom in patients was epigastric pain, accounting for 65.9%.

3.2. The *oipA* "on/off", *babA2, cagE and cagA* GENES OF *HELICOBACTER PYLORI* IN PATIENTS WITH CHRONIC GASTRITIS AND PEPTIC ULCER DISEASE

3.2.1. The oipA gene *of H. pylori*

3.2.1.1. The percentage of the oipA gene "on/off" status

The proportion of *H. pylori* strains carrying the *oipA* "on" gene accounted for 96.0%, and “off” was 4.0%.

3.2.1.2. The CT repeat patterns of the oipA gene

There were a total of 25 *CT repeat patterns* (19 "on" and 6 "off"). In the *oipA* "on" gene, the "2+1+1+1" (31.8%) and "3+1" patterns (26.0%) were common. There were 5 new CT repeat patterns identified in the *oipA* "on" gene.

3.2.2. The babA2* gene *of H. pylori

The proportion of *H. pylori* strains carrying the *babA2 (+)* gene accounted for 74.6%.

3.2.3. The cagE and cagA genes of H. pylori

The proportion of *H. pylori* strains carrying the *cagE (+) or cagA (+)* gene accounted for 83.8%.

3.2.4. The association between the genes of oipA “on/off”, babA2, cagE, and cagA của H. pylori

There was a significant association between the *cagA* gene and the *babA2*, *oipA* "on/off", and *cagE* genes ($p < 0.05$). There was no association between the *oipA* "on/off" gene and the *babA2* gene ($p > 0.05$).

3.2.5. The association between the genes of oipA “on/off”, babA2, cagE, and cagA of H. pylori and age group and gender

There was no association between the genes of *oipA* “on/off”, *babA2, cagE, and cagA* of *H. pylori* and age group and gender ($p > 0.05$).

3.3. THE ASSOCIATION BETWEEN GENES OR GENE COMBINATIONS OF *oipA “on/off”, babA2, cagE and cagA* OF *HELICOBACTER PYLORI* AND CHRONIC GASTRITIS AND PEPTIC ULCER DISEASE

3.3.1. The association between each gene of oipA "on/off", babA2, cagE, and cagA of H. pylori and chronic gastritis and peptic ulcer disease

3.3.1.1. The association between the oipA "on/off" and chronic gastritis and peptic ulcer disease

Table 3.15. The association between the *oipA* "on/off" gene and chronic gastritis and peptic ulcer disease in logistic regression analysis

Gene	**PU vs. CGNPL**		**CGWPL vs. CGNPL**	
oipA	**OR (95%CI)**	**p***	**OR (95%CI)**	**p**
"off"	1		1	
"on"	13.96 (1.49-1856.39)	**0.016***	6.89 (1.28-37.27)	**0.025**

Notes: chronic gastritis with non-precancerous lesions (CGNPL) was the reference group. CGWPL: chronic gastritis with precancerous lesions, PU: peptic ulcers. Analyzing using Firth logistic regression with the "logistf" package in R*

The *oipA* "on" gene of *H. pylori* was associated with a 13.96-fold increased risk of PU and a 6.89-fold increased risk of CGWPL.

3.3.1.2. The association between the babA2 gene and chronic gastritis and peptic ulcer disease

Table 3.17. The association between the *babA2* gene and chronic gastritis and peptic ulcer disease in logistic regression analysis

babA2 **gene**	**PU vs. CGNPL**		**CGWPL vs. CGNPL**	
	OR (95%CI)	**p**	**OR (95%CI)**	**p**
babA2 (-)	1		1	
babA2 (+)	0.89 (0.33 – 2.39)	0.822	1.15 (0.48 – 2.74)	0.748

Notes: chronic gastritis with non-precancerous lesions (CGNPL) was the reference group. CGWPL: chronic gastritis with precancerous lesions, PU: peptic ulcers

There was no association between the *babA2* (+) gene and PU or CGWPL.

3.3.1.3. The association between the cagA, cagE gene and gene combination cagA/ cagE and chronic gastritis and peptic ulcer disease

Table 3.19. The association between the *cagA, cagE* gene, and *cagA/ cagE* and chronic gastritis and peptic ulcer disease in logistic regression analysis

Gene	PU vs. CGNPL		CGWPL vs. CGNPL	
	OR (95%CI)	**p**	**OR (95%CI)**	**p**
Gene cagA *cagA (-)*	1		1	
cagA (+)	8,15 (1.67-39.71)	**0.009**	2.12 (0.87-5.17)	0.099
Gene cagE *cagE (-)*	1		1	
cagE (+)	8.15 (1.67-39.71)	**0.009**	2.12 (0.87-5.17)	0.099
cagA/ cagE *Other cagA/ cagE*	1		1	
cagA (+)/ *cagE* (+)	8.15 (1.67-39.71)	**0.009**	1.82 (0.76-4.38)	0.180

Notes: chronic gastritis with non-precancerous lesions (CGNPL) was the reference group. CGWPL: chronic gastritis with precancerous lesions, PU: peptic ulcers

The *cagA (+), cagE (+), or cagA* (+)/ *cagE* (+) of *H. pylori* was associated with an 8.15-fold increased risk of PU, but not associated with CGWPL.

3.3.2. The association between the gene combinations of oipA, babA2, cagE và cagA and chronic gastritis and peptic ulcer disease

3.3.2.1. The association between the cagA/ oipA and chronic gastritis and peptic ulcer disease

Table 3.22. The association between the *cagA/ oipA* of *H. pylori* and chronic gastritis and peptic ulcer disease in logistic regression analysis

cagA/ oipA	**PU vs. CGNPL**		**CGWPL vs. CGNPL**	
	OR (95%CI)	**p**	**OR (95%CI)**	**p**
Other cagA/ oipA	1		1	
cagA (+)/*oipA* "bật"	11.67 (2.44 – 55.83)	**0.002**	3.03 (1.28 – 7.17)	**0.011**

Notes: chronic gastritis with non-precancerous lesions (CGNPL) was the reference group. CGWPL: chronic gastritis with precancerous lesions, PU: peptic ulcers

The *cagA* (+)/*oipA* "on" of *H. pylori* was associated with an 11.67-fold increased risk of PU and a 3.03-fold increased risk of CGWPL.

3.3.2.2. The association between the cagA/ cagE/ oipA and chronic gastritis and peptic ulcer disease

Table 3.28. The association between the *cagA/cagE/oipA* and chronic gastritis and peptic ulcer disease in logistic regression analysis

cagA/ cagE/ oipA	**PU vs. CGNPL**		**CGWPL vs. CGNPL**	
	OR(95%CI)	**p**	**OR (95%CI)**	**p**
OthercagA/*cagE*/*oipA*	1		1	
cagA (+)/*cagE* (+)/ *oipA* "bật"	11,67 (2.44- 55.83)	**0.002**	2.61 (1.12 -6.06)	**0.026**

Notes: chronic gastritis with non-precancerous lesions (CGNPL) was the reference group. CGWPL: chronic gastritis with precancerous lesions, PU: peptic ulcers

The *cagA* (+)/ *cagE* (+)/ *oipA* "on" of *H. pylori* was associated with an 11.67-fold increased risk of peptic ulcers and a 2.61-fold increased risk of chronic gastritis with precancerous lesions.

3.3.2.3. The association between the cagA/ babA2, oipA/ babA2, cagA/ cagE/ babA2 and chronic gastritis and peptic ulcer disease

There was no association between gene combinations of *cagA (+)/ babA2 (+), oipA "on"/ babA2(+), cagA(+)/ cagE(+)/ babA2(+) with* peptic ulcers or chronic gastritis with precancerous lesions (p>0.05).

Chapter 4: DISCUSSION

4.1. General Characteristics of the Study Sample

4.1.1. Age, Gender, Smoking

The mean age was 42.6 ± 15.1, higher than Dang Ngoc Quy Hue'study (2018). The age group ≥40 was 53.2%, with males comprising 49.1%, similar to the findings of Nguyen Thi Mai Ngan (2021). 20.2% of patients were smokers, higher than the findings of Dang Ngoc Quy Hue (2018).

4.1.2. The characteristics of chronic gastritis and peptic ulcer disease

4.1.2.1. The distribution of chronic gastritis and peptic ulcer disease

The rate of peptic ulcer (PU) (24.3%) was similar to Nguyen Thi Mai Ngan's (24.3%) and lower than Ha Thi Minh Thi's (28.1%). The percentage of chronic gastritis with precancerous lesions (CGWPL) was 53.7%, relatively high and similar to the findings of Nguyen Quang Chung's study (2007).

4.1.2.2. The association between age group and gender and chronic gastritis and peptic ulcer disease

There was an association between age group and gender with chronic gastritis and peptic ulcer disease. Patients in the age group ≥40 and males had a higher rate in the PU group, consistent with Nguyen Thi Mai Ngan (2021) and Farzi (2018).

4.1.3. The clinical symptoms

The epigastric pain is the most common symptom, similar to findings by Dang Ngoc Quy Hue (2018) and Nguyen Thi Mai Ngan (2021).

4.2. The *oipA* "on/off", *babA2, cagE and cagA* GENES OF *HELICOBACTER PYLORI*

4.2.1. The oipA gene *of H. pylori*

4.2.1.1. The percentage of the oipA gene "on/off" status

The prevalence of the *oipA* "on" gene of *H. pylori* was 96%, similar to studies in East Asia reporting over 90%, such as Zhao (2020) in China (100%) and Ando (2002) in East Asian countries (100%). However, this result is higher than some studies in Western countries, such as Markovska (2011) in Bungary (81%), Yanovich (2023) in Belarus (79.8%). This indicates geographical variations in the prevalence of *oipA* "on" gene of *H. pylori*.

4.2.1.2. The CT repeat patterns of the oipA gene

The 19 CT repeat patterns of the *oipA* "on" gene were noted, higher than that of studies in other East Asian and Western countries, which may be due to geographical genetic variations of *H. pylori*. The most common CT repeat patterns of the *oipA* "on" gene are "2 + 1 + 1 + 1" and "3 + 1", consistent with other studies in East Asia, different from studies in Western countries. Furthermore, five new CT repeat patterns of the *oipA* "on" gene of *H. pylori* were noted. Our results noted geographical differences in CT repeat patterns of the *oipA* "on" gene.

4.2.2. The babA2 gene *of H. pylori*

The rate of the *babA2* (+) gene is 74.6%, lower than some studies in Asia, such as Lee's study (2021) in South Korea (70.1%) or Azizimoghaddam's study (2023) in Iran (97.1%). However, the results are higher than Bucci's study (2023) in Argentina (53%) or Molina-Castro's study (2019) in Costa Rica (44%). The results show the rate of *babA2* (+) gene in Vietnam, and the molecular characteristics of *H. pylori* that differ geographically.

4.2.3. The cagE and cagA genes of H. pylori

** The cagE gene*

The proportion of the *cagE* (+) gene is 83.8%, higher than GholizadeTobnagh's study (2017) in Iran (69.4%), and lower than Chomvarin's study (2008) in Thailand (88.4%). However, the results are higher than El Khadir's study (2021) in Morocco (55%) or Boyanova's study (2011) in Bungary (68,5%). The initial results indicate a high proportion of the *cagE* (+) gene in *H. pylori* strains in Vietnam.

** The cagA gene*

The rate of the *cagA* (+) gene is 83.8%, similar to Tserentogtokh's study (2019) in Mongolia (82.3%) but lower than Xue's study (2020) in China (97%). Besides, our result is higher than Idowu's study (2019) in South Africa (32.6%) or Kishk's study (2019) in Egypt (53%). Compared with some domestic studies, the results are similar to Phan Trung Nam's study (2017) in Hue (84%), and higher than Nguyen Hoa Trang (2021) at Ho Chi Minh city (79.5%). The difference in the rate

of the *cagA* (+) gene among regions in Vietnam demonstrates the high genetic diversity of *H. pylori*.

4.2.4. The association between the genes of oipA "on/off", babA2, cagE, and cagA của H. pylori

The study noted a significant relationship between the *cagA* gene and the *oipA*, *babA2*, and *cagE* genes of *H. pylori*, similar to findings by Dabiri (2017) and Farzi (2018). No association between the *babA2* (+) gene and the *oipA* "on" gene was observed, differing from Dossumbekova (2006). This difference may be due to geographical variations in the molecular characteristics of *H. pylori* and further larger-scale studies are needed to understand better the relationship between these two adhesin-encoding genes of *H. pylori* in Vietnam.

4.2.5. The association between the genes of oipA "on/off", babA2, cagE, and cagA of H. pylori and age group and gender

We evaluated the relationship between the *oipA*, *babA2*, *cagE*, and *cagA* genes of *H. pylori* with the age groups and gender to identify the patient population at risk of infection with highly virulent strains of *H. pylori*. However, this relationship has not been observed in the study.

4.3. THE ASSOCIATION BETWEEN GENES OR GENE COMBINATIONS OF *oipA "on/off", babA2, cagE and cagA* OF *HELICOBACTER PYLORI* AND CHRONIC GASTRITIS AND PEPTIC ULCER DISEASE

4.3.1. The association between each gene of oipA "on/off", babA2, cagE, and cagA of H. pylori and chronic gastritis and peptic ulcer disease

4.3.1.1. The association between the oipA "on/off" gene and chronic gastritis and peptic ulcer disease

The results showed that the *oipA* "on" gene increased the risk of PU by 13.96 times (95% CI: 1.5-1856.39), consistent with the study by Yanovich (2022) but different from Singh's (2023). The relationship between *oipA* "on" gene of *H. pylori* and PU remains unclear. Although the wide range of the 95% CI of the odds ratio, explained by the relatively small sample size and univariate analysis in our study, these initial results suggest an association between the *oipA* "on" gene of *H. pylori* and an increased risk of PU, providing a basis for larger-scale studies on the *oipA* "on/off" gene of *H. pylori* strains in Vietnam.

Furthermore, the *oipA* "on" gene was found to be associated with an increased risk of CGWPL, differing from the findings of Farzi (2018) and Zhao (2020). This difference could be attributed to geographical variations. Therefore, larger-scale research is needed to elucidate further the relationship between the *oipA* "on" gene and the risk of CGWPL in Vietnam.

4.3.1.2. The association between the babA2 gene and chronic gastritis and peptic ulcer disease

The study found no association between the *babA2*(+) gene and the risk of PU or CGWPL, consistent with Chen (2013) and Molina-Castro (2019). However, the results differ from Askari (2021) or Yu (2002). Currently, the relationship between *babA2* gene of *H. pylori* and gastroduodenal diseases remains inconsistent. Therefore, our preliminary results may contribute to understanding the role of the *babA2* gene in *H. pylori* strains in the development of gastroduodenal diseases in Vietnam.

4.3.1.3. The association between the cagA, cagE gene, and gene combination of cagA/ cagE and chronic gastritis and peptic ulcer disease

* *cagE gene*

The results indicated that the *cagE*(+) gene increases the risk of PU, similar to Khatoon (2017) in India but different from GholizadeTobnagh (2016) in Iran. Our study did not find an association between the *cagE* gene and CGWPL.The relationship between the *cagE*(+) gene and the risk of CGWPL has not been documented, with only a few studies reporting an association with an increased risk of gastric cancer but lacking inconsistency. Further large-scale studies are needed to understand better the relationship between the *cagE*(+) gene of *H. pylori* and the development of CGWPL in Vietnam.

* *cagA gene*

The results showed that the *cagA* (+) gene increases the risk of PU, similar to Sahara (2012) but different from the study by Xue (2021) in China and Nguyen Thi Mai Ngan (2021) in Hue City. The study did not find an association between the *cagA* (+) gene and CGWPL, similar to Farzi (2018) in Iran. Overall, the relationship between the *cagA*(+) gene of *H. pylori* and PU and CGWPL remains inconsistent, particularly in Asian countries with a high rate of the *cagA*(+) gene.

4.3.2. The association between the gene combinations of oipA, babA2, cagE, and cagA and chronic gastritis and peptic ulcer disease

4.3.2.1. The association between the gene combination of cagA/ oipA and chronic gastritis and peptic ulcer disease

The study found that the *cagA*(+) and *oipA* "on" increased the risk of PU by 11.67 times, higher than the *cagA*(+) gene (8.15 times). Additionally, the results also observed that the gene combination of the *cagA*(+) and *oipA* "on" increased the risk of CGWPL by 3.03 times, while no association between the *cagA*(+) gene and CGPLW. Therefore, our preliminary results indicate a synergistic effect of the two genes of *cagA*(+) and *oipA* "on" in the development of PU and CGWPL.

4.3.2.2. The association between the gene combination of cagA/ cagE/ oipA and chronic gastritis and peptic ulcer disease

The results indicate that the *cagA*(+)/*cagE*(+)/*oipA* "on" increased the risk of PU by 11.67 times, higher than the *cagA*(+) or *cagE*(+) genes (8.15 times). Additionally, it increased the risk of CGWPL by 2.61 times, while no association between the *cagA*(+) or *cagE*(+) genes and CGPLW. Our preliminary findings suggest close coordination of the three genes of *cagA*(+), *cagE*(+), and *oipA* "on," in the development of PU and CGWPL.

4.3.2.3. The association between the gene combination of cagA/ babA2, oipA/ babA2, and cagA/ cagE/ babA2 and chronic gastritis and peptic ulcer disease

The study did not find any association between the gene combinations of *cagA*(+)/*babA2*(+), *oipA*"on"/*babA2*(+), *cagA*(+)/*cagE*(+)/*babA2*(+) and PU or CGWPL. Further large-scale studies are needed to investigate the relationship between these gene combinations and gastroduodenal diseases.

THE LIMITATION OF STUDY

Due to limitations in the study conditions, we only take two gastric biopsies from patients with chronic gastritis for

histopathological examinations. Besides, we did not evaluate the types of intestinal metaplasia or record high-grade dysplasia cases. Therefore, we have not assessed the frequency or relationship between oipA, *babA2, cagE, and cagA* genes and incomplete intestinal metaplasia or high-grade dysplasia.

CONCLUSIONS

1. The genes and the gene combinations of *oipA* "on/off", *babA2, cagE,* and *cagA* of *Helicobacter pylori* in patients with chronic gastritis and peptic ulcer disease

The rate of the *oipA* "on" gene was 96%. A total of 25 CT repeat patterns, with 19 patterns of the *oipA* "on" gene, with the most common patterns being "2+1+1+1" and "3+1". Five new CT repeat patterns of the *oipA* "on" gene were identified.

The proportion of the *babA2* (+) gene was 74.6%.

The proportion of the *cagA* (+) gene was 83.8%.

The proportion of the *cagE* (+) gene was 83.8%.

The proportion of the gene combination of cagA (+)/ cagE (+)/ *oipA* "on"/ *babA2* (+) was 67.6%.

2. The association between each gene and the gene combinations of *oipA* "on/off," *babA2, cagE, and cagA* of *Helicobacter pylori* and chronic gastritis and peptic ulcer disease

2.1. The association between each gene of oipA "on/off", babA2, cagE, and cagA of H. pylori and chronic gastritis and peptic ulcer disease

- The *oipA* "on" gene of *H. pylori* was associated with a 13.96-fold increased risk of peptic ulcers (95%CI: 1,5-1856,39) and a

6.89-fold increased risk of chronic gastritis with precancerous lesions (95%CI: 1,28-37,27).

- There was no association between the *babA2* (+) gene and peptic ulcers or chronic gastritis with precancerous lesions.

- The *cagA (+)* gene of *H. pylori* was associated with an 8.15-fold increased risk of peptic ulcers (95%CI: 1,67-39,71), but not associated with chronic gastritis with precancerous lesions.

- The *cagE (+)* gene of *H. pylori* was associated with an 8.15-fold increased risk of peptic ulcers (95%CI: 1,67-39,71), but not associated with chronic gastritis with precancerous lesions.

2.2. The association between the gene combinations of oipA, babA2, cagE và cagA and chronic gastritis and peptic ulcer disease

- The *cagA (+)/ cagE (+)* gene combination of *H. pylori* was associated with an 8.15-fold increased risk of peptic ulcers (95%CI: 1,67-39,71), but not associated with chronic gastritis with precancerous lesions.

- The *cagA (+)/ oipA "on"* gene combination of *H. pylori* was associated with an 11.67-fold increased risk of peptic ulcers (95%CI: 2.44- 55.83) and a 3.03-fold increased risk of chronic gastritis with precancerous lesions (95%CI: 1.28 – 7.17).

-The *cagA (+)/ cagE (+)/ oipA "on"* gene combination of *H. pylori* was associated with an 11.67-fold increased risk of peptic ulcers (95%CI: 2.44- 55.83) and a 2.61-fold increased risk of chronic gastritis with precancerous lesions (95%CI: 1.12 -6.06).

- There was no asscociation between gene combinations of *cagA (+)/ babA2 (+), oipA "on"/ babA2(+), cagA(+)/ cagE(+)/*

babA2(+) and peptic ulcers or chronic gastritis with precancerous lesions.

RECOMMENDATIONS

We suggest continuing large-scale studies on the virulence genes of *H. pylori*, especially genes encoding outer membrane proteins and other *cag* pathogenicity island genes, to understand better the prevalence of these virulence genes in *H. pylori* strains in Vietnam. Additionally, it is essential to expand the study population to include gastric cancer patients and patients with incomplete intestinal metaplasia or high-grade dysplasia to investigate the relationship between these virulence genes and these lesions.

Based on the research results, in medical facilities equipped to perform molecular biology tests, when identifying patients infected with *H. pylori* strains to carry the *cagA* (+)/ *oipA* "on" or *cagA* (+)/ *cagE* (+)/ *oipA* "on" gene combinations, aggressive eradication of *H. pylori* should be pursued to prevent the progression of peptic ulcer disease or precancerous gastric lesions.

THE PUBLISHED ARTICLES RELATED TO THE STUDY

1. Thai Thi Hong Nhung, Nguyen Thai Hoa, Nguyen Thi Mai Ngan, Ha Thi Minh Thi (2023), "The prevalence of the cagE gene of Helicobacter pylori and its association with gastroduodenal diseases", *Hue Journal of Medicine and Pharmacy*, Volume 13, no. 5, pp. 14-19.

2. Thi Hong Nhung Thai, Hong Phong Nguyen, Thi Hai Yen Nguyen, Thi Be Hai Nguyen, Thai Hoa Nguyen, Thi Mai Ngan Nguyen, Thi Minh Thi Ha (2023), "Genetic diversity of the *oipA* gene among *Helicobacter pylori* isolates and clinical outcome in Vietnam", *Infection, Genetics and Evolution*, 112, 105438. doi: 10.1016/j.meegid.2023.105438.

3. Thai Thi Hong Nhung, Nguyen Thai Hoa, Nguyen Hong Phong, Nguyen Thi Hai Yen, Nguyen Thi Be Hai, Nguyen Thi Mai Ngan, Ha Thi Minh Thi (2023), "The prevalence of the cagA gene of Helicobacter pylori and its association with gastroduodenal diseases", *Can Tho Journal of Medicine and Pharmacy*, 67, pp.13-19.