

## Research Article

# Precancerous Gastric Lesions with *Helicobacter pylori* *vacA*<sup>+</sup>/*babA2*<sup>+</sup>/*oipA*<sup>+</sup> Genotype Increase the Risk of Gastric Cancer

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**Objective.** The clinical outcomes of gastric diseases such as chronic gastritis, peptic ulcer, and gastric cancer have been attributed to the interplay of virulence factors of *Helicobacter pylori* (*H. pylori*), host genetic susceptibility, and host immune responses. This study investigated the presence of *cagA*, *vacA*, *iceA2*, *babA2*, and *oipA* genes and their association with clinical outcomes. **Methods.** Chronic gastritis, atrophic gastritis, and intestinal metaplasia specimens were obtained from patients who underwent endoscopy and surgical resection between January 2017 and December 2018; specimens from gastric cancer patients treated between January 2014 and December 2018 were also added. *H. pylori* infection and virulence genes (*cagA*, *vacA*, *iceA2*, *babA2*, and *oipA*) were determined using real-time PCR. The association between *H. pylori* genotypes and clinical outcomes were evaluated using multivariate regression model analysis. The overall survival of gastric cancer patients was compared between genotype combinations. **Results.** *H. pylori* was positive in 166 patients with chronic gastritis, precancerous gastric lesions, and gastric cancer. The genes *vacA*, *babA2*, and *oipA* were most prevalent in chronic gastritis (73%), precancerous gastric lesions (62%), and gastric cancer (91%), respectively. The *vacA*, *babA2*, and *oipA* genes were associated with increased risk of gastric cancer (OR = 1.23; 95% CI = 1.13–3.32; *P* = 0.033, OR = 2.64; 95% CI = 1.44–4.82, *P* = 0.024, and OR = 2.79; 95% CI = 1.58–5.41; *P* = 0.031, respectively). Interestingly, *H. pylori vacA*<sup>+</sup>/*babA2*<sup>+</sup>/*oipA*<sup>+</sup> genotype infection was associated with increased risk of gastric cancer (OR = 3.85, 95% CI = 1.67–5.77, *P* = 0.014). **Conclusion.** In this present study, we reported on the virulence genes of *H. pylori* infection to reveal their association with increased risk of chronic gastritis, precancerous gastric lesions, and gastric cancer. Precancerous gastric lesions with *H. pylori vacA*<sup>+</sup>/*babA2*<sup>+</sup>/*oipA*<sup>+</sup> genotype increased the risk of gastric cancer.

## 1. Introduction

*Helicobacter pylori* (*H. pylori*) is a spiral-shaped Gram-negative bacterium that selectively colonizes the gastric mucosa of the humans in more than half of the world's population [1]. *H. pylori* infection has been proven to be highly associated with the development of a variety of

gastric diseases such as chronic gastritis, peptic ulcer disease (PUD), mucosal associated lymphoid tissue (MALT), and gastric cancer (GC) [2–4]. These different clinical outcomes have been attributed to the interplay of several factors, including virulence factors of *H. pylori*, host genetic susceptibility, and host immune responses to *H. pylori* infection [5–7]. Several virulence factors have been

proposed for *H. pylori* infections; they include adapting to different tissues like urease and flagella, using adhesins such as blood-antigen binding protein A (BabA) and outer inflammatory protein A (OipA), and toxins that damage host tissues such as cytotoxin-associated gene A (CagA) and vacuolating cytotoxin (VacA). Another putative virulence factor is that induced by contact with epithelium A (IceA) [8, 9].

The BabA is a protein for Lewis b binding activity on human gastric epithelial cells. Three *bab* alleles; *babA1*, *babA2*, and *babB* genes have been identified, but only the *babA2* gene product is functional [10, 11]. BabA2 is associated with an increased risk of PUD and GC [12–14]. The OipA is one of the porin proteins and proinflammatory proteins associated with severe neutrophil infiltration in IL-8 induction and gastric colonization [15]. It contributes to the pathogenesis and is associated with the elevated risks of PUD and GC [16–18]. The *cagA* gene is located at the end of the *cag* pathogenicity island (*cagPAI*), encodes a type IV secretion system (T4SS) that is functional for translocating bacterial effectors into host cytoplasm, and triggers the manipulation of cell signaling pathways and also the induction of the proinflammatory cytokines, specifically interleukin IL-8 [19, 20]. It is associated with severe clinical diseases in PUD and GC [21–24]. The VacA is a pore-forming toxin, which causes progressive vacuolation and injury to the gastric epithelium [25]. It is associated with an increased risk of PUD and GC [26, 27]. Specific allelic types in the *vacA* gene are signal (*s1*, *s2*) and the middle regions (*m1*, *m2*) due to sequence heterogeneity [28]. The variation in cytotoxic activities in relation to *H. pylori*-related diseases and gastric mucosal changes is considered the cause of different strains [29]. The *iceA* gene is induced by contact with epithelium and has been considered as a marker for PUD [30]. It has two main allelic variants, *iceA1* and *iceA2* [31]. It has been found that *iceA1* is the predominant subtype in East Asia, while *iceA2* is the predominant subtype in the USA and Columbia [32]. Although several studies have reported different results, the *iceA2* gene was detected to be the predominant genotype [33, 34].

In intestinal types of GC, *H. pylori* infection triggers a multistep progression from chronic gastritis, atrophic gastritis (AG), intestinal metaplasia (IM), and finally to GC [35]. Several studies suggest that AG and IM are the major precursor gastric lesions of intestinal-type GC and elevate the risk of GC [36–38]. Moreover, AG and IM increased the risk of intestinal-type GC exponentially when compared with other risk factors [39]. From this background, *H. pylori* infection is thought to be involved in chronic gastritis, precancerous gastric lesions, and GC; however, the relationship between virulence status and its association with these clinical outcomes has not been well reported and are not fully understood in Asian countries. Thus, the aim of this study was to investigate the *H. pylori* virulence genes including *cagA*, *vacA*, *iceA2*, *babA2*, and *oipA* of patients with chronic gastritis, precancerous gastric lesions, and GC, and to determine whether the virulence genes are associated with the risk of chronic gastritis, precancerous gastric lesions, and GC.

## 2. Material and Methods

**2.1. Patients and Specimens.** Patients were subjected to esophagogastroduodenoscopy (EGD) (Olympus Corp., Tokyo, Japan), which was carried out using an upper GI video endoscope (Olympus EVIS EXERA III, CV-190). Gastric tissue biopsies of chronic gastritis, AG, and IM were obtained between January 2017 and December 2018, and GC biopsies were obtained between January 2014 and December 2018. Surgical resection was performed at the Suranaree University of Technology Hospital, Buriram Hospital Medical center, or Surin Hospital Medical center in the Northeastern region of Thailand. Written informed consent was obtained from all patients, and the study protocol was approved by the Ethics Committee for Research Involving Human Subjects, Suranaree University of Technology (EC-59-45 and EC 16-2560). The whole stomach was examined and biopsies were conducted using the site-specific biopsy technique [40]. All biopsies were directly tested for *H. pylori* infection by using the rapid urease test (RUT) kit (Pentland Medical, Edinburgh, UK). The methods were carried out in accordance with good clinical practice and the guidelines of the Declaration of Helsinki [41]. Histological determinations were subsequently examined by a pathologist. The patient retrospective cohort included 70 cases, which were used to analyze the association between overall survival (OS) and genotype combinations.

**2.2. DNA Extraction.** DNA extraction from fresh tissues of chronic gastritis, AG, and IM was performed using the QIAamp DNA mini kit (Qiagen, Düsseldorf, Germany), and the tissues of GC were formalin-fixed and paraffin-embedded (FFPE) using xylene and hydrate in 100% ethanol and subsequently by using QIAamp DNA FFPE tissue kit (Qiagen, Düsseldorf, Germany) according to the manufacturer's instructions. Genomic DNA was purified from the tissue lysate using the QIAamp spin column and eluted. The DNA concentration and purity were determined using a DS-11+ spectrophotometer (Denovix, Wilmington, Delaware, USA) and stored at  $-20^{\circ}\text{C}$ .

**2.3. Real-Time Polymerase Chain Reaction (Real-Time PCR).** *H. pylori* infection 16s *rRNA* and *ureA* genes were identified. *H. pylori*-positive samples were used to determine the virulence genes (*cagA*, *vacA*, *iceA2*, *babA2*, and *oipA*) using real-time PCR. The primers for 16s *rRNA*, *ureA*, *cagA*, *vacA*, *iceA2*, *babA2*, and *oipA* (Integrated DNA Technologies, Coralville, IA, USA) are shown in Table 1. Briefly, DNA samples were used as templates in the amplification reactions. The real-time PCR was performed according to the manufacturer's protocol in a final volume of 20  $\mu\text{L}$  containing DNA template, 2X SYBR Green PCR Master Mix (Roche Applied Science, Mannheim, Germany), and 50 pmol of each primer using a Light Cycler<sup>®</sup> 480 Instrument (Roche diagnostics, Neuilly sur Seine, France). The PCR conditions used in this study were as follows: pre-incubation at  $95^{\circ}\text{C}$  for 5 min, 45 cycles of amplification (10 s of denaturation at  $95^{\circ}\text{C}$ , 10 s of annealing at  $T_m$  of specific

TABLE 1: Primers used for the amplification of *H. pylori* genes.

Primer	Forward	Reverse	Reference
16s rRNA	GGAGTACGGTCGCAAGATTTAAA	CTAGCGGATTCTCTCAATGTCAA	[42]
UreA	CGTGGCAAGCATGATCCAT	GGGTATGCACGGTTACGAGTTT	[43]
CagA	GAGTCATAATGGCATAGAACCTGAA	TTGTGCAAGAAATTCATGAAA	[44]
VacA	CTCCAGAAGGCACACCAATAA	TGGCTTCCACTTCCCCATTAA	[45]
IceA2	GTTGTCGTTGTTTTAATGAA	GTCTTAAACCCCACGATTTAA	[46]
BabA2	CCAAACGAAACAAAAAGCGT	GCTTGTGTA AAAAGCCGTCGT	[47]
OipA	GTTTTTGATGCATGGGATTT	GTGCATCTCTTATGGCTTT	[48]

primer, and 10 s of extension at 72°C). Each sample was performed in duplicates reactions for standard. All data were analyzed using the Light Cycler 480 software, version 1.5 (Roche diagnostics, Neuilly sur Seine, France).

**2.4. Statistical Analysis.** The differences between the virulence genes of *H. pylori* infection for the patient's demographic data were determined using ANOVA. The associations between virulence genes and clinical outcomes and risks of GC were evaluated using the univariate regression model analysis. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using the multivariate regression model analysis. Survival analysis was performed using the Kaplan–Meier method and the overall survival differences were analyzed using the log-rank test. A *P* value of less than 0.05 was considered statistically significant. All statistical analyses were carried out using SPSS for Windows (version 20.0; IBM Corp., Armonk, NY, USA).

### 3. Results

**3.1. Detection of *H. pylori* cagA, vacA, iceA2, babA2, and oipA Virulence Genes in Chronic Gastritis, Precancerous Gastric Lesions, and Gastric Cancer.** A total of 200 *H. pylori*-positive samples was examined for 16s rRNA and ureA gene detection; out of which 166 patients (83%) were positive for both 16s rRNA and ureA genes. These patients were divided into three groups: chronic gastritis (*n* = 44), precancerous gastric lesions including AG and IM (*n* = 52), and GC (*n* = 70). All patients were not significantly different in age and gender. The patient's demographic data are summarized in Table 2. The vacA (73%) gene was mainly present in chronic gastritis followed by cagA (68%), babA2 (59%), oipA (27%), and iceA2 (9%). The babA2 (62%) gene was mainly present in precancerous gastric lesions followed by cagA and oipA (46%), vacA (27%), and iceA2 (19%). Interestingly, babA2 and oipA (91%) were almost present in GC followed by vacA (60%), cagA (26%), and iceA2 (16%). The iceA2 gene was the lowest frequency of detection in all clinical outcomes. The frequency of cagA, vacA, iceA2, babA2, and oipA genes is shown in Figure 1. The presence of vacA, babA2, and oipA genes was significantly different between clinical outcomes (*P* = 0.036, 0.042 and 0.039, respectively).

**3.2. Association between the Presence of vacA, babA2, and oipA Genes and Clinical Outcomes.** The association between the presence of vacA, babA2, and oipA genes and clinical

outcomes was assessed. Among chronic gastritis, vacA was present in *H. pylori* infection and was associated with significantly increased risk of chronic gastritis (OR = 2.14, 95% CI = 1.62–4.46, *P* = 0.036). The vacA, babA2, and oipA genes were present in patients, but they were not associated with increased risk of precancerous gastric lesions. Additionally, vacA, babA2, and oipA genes were associated with increased risk of GC (OR = 1.23; 95% CI = 1.13–3.32; *P* = 0.033, OR = 2.64; 95% CI = 1.44–4.82, *P* = 0.024 and OR = 2.79; 95% CI = 1.58–5.41; *P* = 0.031, respectively) (Table 3).

**3.3. *H. pylori* vacA<sup>+</sup>/babA2<sup>+</sup>/oipA<sup>+</sup> Genotype Conferred Increased Risk of GC.** We examined the virulence combinations based on the analysis of vacA, babA2, and oipA genotypes. Precancerous gastric lesions infected with vacA<sup>+</sup>/babA2<sup>+</sup>, vacA<sup>+</sup>/oipA<sup>+</sup>, babA2<sup>+</sup>/oipA<sup>+</sup>, and vacA<sup>+</sup>/babA2<sup>+</sup>/oipA<sup>+</sup> genotypes were 3.85%, 3.85%, 26.92%, and 11.54%, whereas GC were 2.86%, 2.86%, 34.29%, and 51.43%, respectively (Table 4). Interestingly, precancerous gastric lesions infected with *H. pylori* genotype combination of vacA<sup>+</sup>/babA2<sup>+</sup>/oipA<sup>+</sup> were highly significantly associated with increased risk of GC (OR = 3.85, 95% CI = 1.67–5.77, *P* = 0.021), but not vacA<sup>+</sup>/babA2<sup>+</sup>, vacA<sup>+</sup>/oipA<sup>+</sup>, and babA2<sup>+</sup>/oipA<sup>+</sup> genotypes. Chronic gastritis was not associated with the development of precancerous gastric lesions or GC when infected with any *H. pylori* genotype combination (data not shown).

**3.4. Overall Survival of GC Patients with *H. pylori* Genotype Combination Infections.** We examined the overall survival of GC patients that were infected with *H. pylori* two-genotype strains (vacA<sup>+</sup>/babA2<sup>+</sup>, vacA<sup>+</sup>/oipA<sup>+</sup>, and babA2<sup>+</sup>/oipA<sup>+</sup>) and *H. pylori* three-genotype strain (vacA<sup>+</sup>/babA2<sup>+</sup>/oipA<sup>+</sup>). The mean survival time for patients with *H. pylori* two-genotype infection was 69.52 ± 3.72 months and with *H. pylori* three-genotype infection was 56.16 ± 3.23 months. However, the overall survival of patients infected with vacA<sup>+</sup>/babA2<sup>+</sup>/oipA<sup>+</sup> genotype strain was decreased, but there was no statistically significant difference between the two groups (*P* = 0.148; Figure 2).

### 4. Discussion

Our study was a cross-sectional study, which investigated the presence of cagA, vacA, iceA2, babA2, and oipA genes in *H. pylori* infected patients with chronic gastritis, precancerous gastric lesions, and GC. The rate of *H. pylori* infection

TABLE 2: Demographic characteristics.

	Chronic (n = 44)	Precancerous lesions (n = 52)	Gastric cancer (n = 70)	P value
Age: mean	43 ± 1.6	46 ± 2.4	52 ± 1.2	0.192
Gender: male/female (%)	63.6/32.4	46.2/43.8	45.2/44.8	0.082
Pathological characteristic of gastric cancer patients (n = 70)				
Location of tumor n (%)				
Upper				12 (17.15)
Middle				36 (51.42)
Lower				22 (31.43)
Tumor size n (%)				
<70 mm				18 (25.72)
≥70 mm				52 (74.28)
Histologic type n (%)				
Differentiated				54 (77.14)
Undifferentiated				16 (22.86)
Lymphatic invasion n (%)				
Absent				46 (65.71)
Present				24 (34.29)
Vascular invasion n (%)				
Absent				62 (88.57)
Present				8 (11.43)
Pathological T stage n (%)				
T1-T2				22 (31.43)
T3-T4				48 (68.57)
Pathological TNM stage n (%)				
I				8 (11.43)
II				14 (20.0)
III				36 (51.43)
IV				12 (17.14)
Residual tumor n (%)				
No residual tumor				52 (74.28)
Microscopic				6 (8.57)
Gross (unresectable)				12 (17.14)
CEA n (%)				
<5.0 (ng/ml)				42 (60.0)
≥5.0 (ng/ml)				28 (34.29)

Comparisons between the groups were done by using ANOVA.  $P < 0.05$  considered as statistically significant.

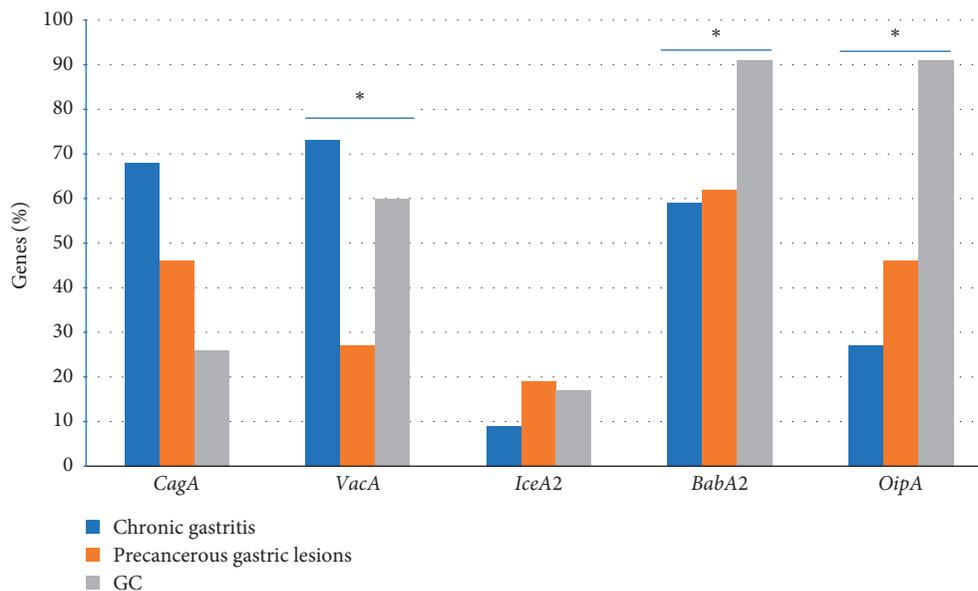


FIGURE 1: The frequency of cagA, vacA, iceA2, baba2, and oipA genes in each clinical outcome.

TABLE 3: Virulence gene in association with clinical outcomes.

Gastric mucosa pathology/ Virulence gene	Chronic (n = 44)	Precancerous (n = 52)	OR; 95% CI	P value	Precancerous (n = 52)	GC (n = 70)	OR (95% CI)	P value
<i>VacA</i>	32 (73%)	14 (27%)	2.14 (1.62–4.46)	0.036	14 (27%)	42 (60%)	1.23 (1.13–3.32)	0.033
<i>BabA2</i>	26 (59%)	32 (62%)	0.77 (0.56–0.94)	0.833	32 (62%)	64 (91%)	2.64 (1.44–4.82)	0.024
<i>OipA</i>	12 (27%)	24 (46%)	0.69 (0.49–0.82)	0.546	24 (46%)	64 (91%)	2.79 (1.58–5.41)	0.031

Multivariate regression model analysis used to analyze the data. OR: odds ratio; CI: confidence interval. Significance is set at  $P < 0.05$ .

TABLE 4: Virulence genotype combination in association with clinical outcomes.

Virulence gene			Precancerous gastric lesion (%)	GC (%)	OR (CI 95%)	P value
<i>VacA</i>	<i>BabA2</i>	<i>OipA</i>				
+	+	-	2 (3.85)	2 (2.86)	0.72 (0.42–0.97)	0.634
+	-	+	2 (3.85)	2 (2.86)	0.72 (0.42–0.97)	0.634
-	+	+	14 (26.92)	24 (34.29)	0.7 (0.37–0.96)	0.091
+	+	+	6 (11.54)	36 (51.43)	4.28 (1.82–7.41)	0.021

Multivariate regression model analysis used to analyze the data. OR: odds ratio; CI: confidence interval. Significance is set at  $P < 0.05$ .

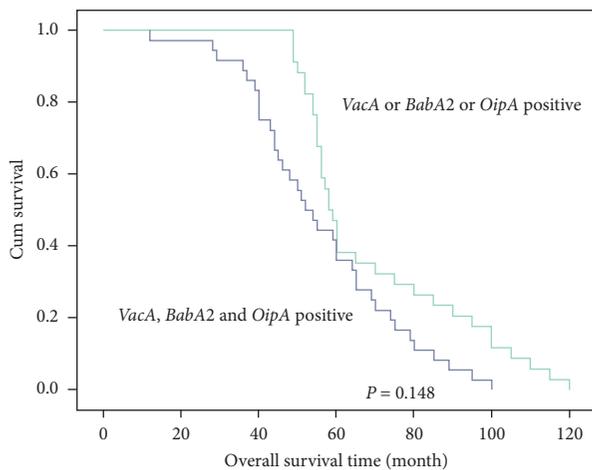


FIGURE 2: Overall survival time of gastric cancer patients infected with *H. pylori* two-genotypes combination ( $vacA^+/babA2^+$ ,  $vacA^+/oipA^+$  and  $babA2^+/oipA^+$ ) and *H. pylori* three-genotypes combination ( $vacA^+/babA2^+/oipA^+$ ).

in this study was 83%, which was comparable with a retrospective study in the Northeastern region of Thailand [49] and a prospective study in Japan [36]. This study was the first report on the associations between virulence genes and the risk of chronic gastritis, precancerous gastric lesions, and GC in Thailand. Our results revealed that the *vacA* gene was associated with chronic gastritis whereas *vacA*, *babA2*, and *oipA* genes were associated with increased risk of GC. These indicated that *vacA* and *babA2* genes influenced chronic gastritis and precancerous gastric lesions, respectively. Meanwhile, the *babA2* and *oipA* genes had virulence potential on GC development. The *babA2* and *oipA* genes were present mostly in *H. pylori*-positive GC; it seems *vacA*, *babA2*, and *oipA* genes exhibited different levels of virulence. It is probable that *vacA* alone was not directly associated with gastric carcinogenesis. Although, *vacA* effects on

disruption of gastric epithelial barrier function and modulation of the inflammatory response, *vacA* also suppresses the activation of ERK1/2 mitogen-activated protein (MAP) kinase suggesting that *H. pylori* can avoid the induction of excess cellular damage and maintain long-term colonization [5]. Therefore, *vacA* individual may develop chronic gastritis.

*H. pylori* infection induced cell-mediated immunity. Th1 cells play a central role in *H. pylori* immune response. Predominant Th17 expression was positively correlated with the degree of immunopathologic reactions resulting in peptic ulcers [6]. Furthermore, increasing of T-bet+ cells and the mucosal INF- $\gamma$  expression related to the degree of *H. pylori* density in infected patients can lead to ulcer or GC [7]. In addition, the roles of *babA2* and *oipA* proteins might have the potential to exert pressure on *H. pylori* by enhancing the production of free radicals that cause mutations in target cells and the neoplastic clones are established [39]. Tumor necrosis factor alpha (TNF- $\alpha$ ) plays major roles in the growth, invasion, and metastasis of neoplasm called a perigenetic pathway [50]. The TNF- $\alpha$  inducing protein (Tip $\alpha$ ) from *H. pylori* binds to and enters the nucleus through a specific binding molecule, which acts as a carcinogen [51] and contributes to GC. Although, *cagA* is recognized as an oncoprotein and confers oncogenesis, the presence of *cagA* was not associated with any clinical outcome, suggesting that the *cagA* gene present in East Asian strains might not be influential of its risk enough.

Interestingly, genotype combination was associated with GC. The precancerous gastric lesions with *H. pylori*  $vacA^+/babA2^+/oipA^+$  genotype infection had high association with 4.3-fold increased risk of GC development. The *babA2* gene has been strongly associated with *vacA* and increased risk of GC development [24]; however, in this study, the  $babA2^+/vacA^+$  genotype did not have increased risk of GC. Taken together, *oipA* was associated with higher neutrophil activity and IL-8 secretion and showed toxic effects by an apoptosis-

triggered cascade via signaling that affected the Bax/Bcl-2 protein ratio and cleaved-caspase 3 level, leading to a mitochondrial apoptotic cascade [52–54]. These findings suggest that the *vacA*<sup>+</sup>/*babA2*<sup>+</sup>/*oipA*<sup>+</sup> genotype may contribute to the genotoxicity caused by DNA damage and aberrant methylation of genes in *H. pylori*-related gastric carcinogenesis. A cohort study with long-term follow-up demonstrated that infection with *cagA* genotype was associated with increased risk of precancerous gastric lesions progression [55, 56]. Therefore, *vacA*, *babA2*, and *oipA* have been implicated in the development of GC. Regarding precancerous gastric lesions after *H. pylori* eradication, *H. pylori*-induced chronic inflammation can provide the seed of cascade leading to GC, which can continuously progress even in the absence of *H. pylori* [57]. The patients with *H. pylori* and IM have more than 6.4-fold increased risk of GC than that of the patients with *H. pylori* but without IM [36]. However, 2.9% GC was developed in individuals during the mean follow-up of 7.8 years [36]. Therefore, precancerous gastric lesions patients infected with the *H. pylori vacA*<sup>+</sup>/*babA2*<sup>+</sup>/*oipA*<sup>+</sup> genotype were prone to developing GC compared with patients infected with other combination genotypes. However, in the present study, the overall survival time of GC patients with the *H. pylori vacA*<sup>+</sup>/*babA2*<sup>+</sup>/*oipA*<sup>+</sup> genotype infection was not reduced.

The limitations of the present study were that subanalysis for the precancerous gastric lesions was not performed and the number of patients involved was small and there was no regular follow-up on them. Furthermore, several virulence factors of *H. pylori* were not investigated. The expression of *vacA*, *babA2*, and *oipA* protein should be evaluated in a future study of GC carcinogenesis to determine the underlying mechanisms associated with GC development in precancerous gastric lesions patients.

## 5. Conclusion

This study provided important information regarding the presence of virulence genes in different clinical outcomes of *H. pylori* infection. Precancerous gastric lesions of patients infected with *H. pylori vacA*<sup>+</sup>/*babA2*<sup>+</sup>/*oipA*<sup>+</sup> genotype infection have an increased risk of GC. The *H. pylori vacA*<sup>+</sup>/*babA2*<sup>+</sup>/*oipA*<sup>+</sup> genotype might prove helpful in predicting individuals in the high-risk group of GC in the Thai population.

## Data Availability

No data were used to support this study.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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## References

- [1] A. Covacci, J. L. Telford, G. Del Giudice, J. Parsonnet, and R. Rappuoli, “*Helicobacter pylori* virulence and genetic geography,” *Science*, vol. 284, no. 5418, pp. 1328–1333, 1999.
- [2] D. B. Polk and R. M. Peek Jr., “*Helicobacter pylori*: gastric cancer and beyond,” *Nature Reviews Cancer*, vol. 10, no. 6, pp. 403–414, 2010.
- [3] R. M. Peek Jr. and M. J. Blaser, “*Helicobacter pylori* and gastrointestinal tract adenocarcinomas,” *Nature Reviews Cancer*, vol. 2, no. 1, pp. 28–37, 2002.
- [4] C. Y. Kao, B. S. Sheu, and J. J. Wu, “*Helicobacter pylori* infection: an overview of bacterial virulence factors and pathogenesis,” *Biomedical Journal*, vol. 39, no. 1, pp. 14–23, 2016.
- [5] L. E. Wroblewski, R. M. Peek Jr., and K. T. Wilson, “*Helicobacter pylori* and gastric cancer: factors that modulate disease risk,” *Clinical Microbiology Reviews*, vol. 23, no. 4, pp. 713–739, 2010.
- [6] N. Bagheri, A. Razavi, B. Pourgheysari et al., “Up-regulated Th17 cell function is associated with increased peptic ulcer disease in *Helicobacter pylori*-infection,” *Infection, Genetics and Evolution*, vol. 60, pp. 117–125, 2018.
- [7] N. Bagheri, H. Shirzad, Y. Mirzaei et al., “T-bet+ cells polarization in patients infected with *Helicobacter pylori* increase the risk of peptic ulcer development,” *Archives of Medical Research*, vol. 50, no. 3, pp. 113–121, 2019.
- [8] Y. Yamaoka and D. Y. Graham, “*Helicobacter pylori* virulence and cancer pathogenesis,” *Future Oncology*, vol. 10, no. 8, pp. 1487–1500, 2014.
- [9] Y. Saeidi, A. Pournajaf, M. Gholami et al., “Determination of *Helicobacter pylori* virulence-associated genes in duodenal ulcer and gastric biopsies,” *Medical Journal of the Islamic Republic of Iran*, vol. 31, pp. 555–559, 2017.
- [10] D. T. Pride, R. J. Meinersmann, and M. J. Blaser, “Allelic variation within *Helicobacter pylori* *babA* and *babB*,” *Infection and Immunity*, vol. 69, no. 2, pp. 1160–1171, 2001.
- [11] G. Posselt, S. Backert, and S. Wessler, “The functional interplay of *Helicobacter pylori* factors with gastric epithelial cells induces a multi-step process in pathogenesis,” *Cell Communication and Signaling*, vol. 11, no. 1, 2013.
- [12] M. Miftahussurur and Y. Yamaoka, “*Helicobacter pylori* virulence genes and host genetic polymorphisms as risk factors for peptic ulcer disease,” *Expert Review of Gastroenterology & Hepatology*, vol. 9, no. 12, pp. 1535–1547, 2015.
- [13] L. Boyanova, D. Yordanov, G. Gergova, R. Markovska, and I. Mitov, “Association of *iceA* and *babA* genotypes in *Helicobacter pylori* strains with patient and strain characteristics,” *Antonie Van Leeuwenhoek*, vol. 98, no. 3, pp. 343–350, 2010.
- [14] P. Lehours, A. Menard, S. Dupouy et al., “Evaluation of the association of nine *Helicobacter pylori* virulence factors with strains involved in low-grade gastric mucosa-associated lymphoid tissue lymphoma,” *Infection and Immunity*, vol. 72, no. 2, pp. 880–888, 2004.
- [15] Y. Yamaoka, S. Kikuchi, H. M. T. El-Zimaity, O. Gutierrez, M. S. Osato, and D. Y. Graham, “Importance of *Helicobacter pylori* OipA in clinical presentation, gastric inflammation, and mucosal interleukin 8 production,” *Gastroenterology*, vol. 123, no. 2, pp. 414–424, 2002.
- [16] N. Souod, M. Sarshar, H. Dabiri et al., “The study of the OipA and DupA genes in *Helicobacter pylori* strains and their relationship with different gastroduodenal diseases,” *Gastroenterology and Hepatology from Bed to Bench*, vol. 8, no. Suppl 1, pp. S47–S53, 2015.

- [17] J. Liu, C. He, M. Chen, Z. Wang, C. Xing, and Y. Yuan, "Association of presence/absence and on/off patterns of *Helicobacter pylori* OipA gene with peptic ulcer disease and gastric cancer risks: a meta-analysis," *BMC Infectious Diseases*, vol. 13, p. 555, 2013.
- [18] Y. L. Su, H. L. Huang, B. S. Huang et al., "Combination of OipA, BabA, and SabA as candidate biomarkers for predicting *Helicobacter pylori*-related gastric cancer," *Scientific Reports*, vol. 6, p. 36442, 2016.
- [19] R. A. Alm, L. S. Ling, D. T. Moir et al., "Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*," *Nature*, vol. 397, no. 6715, pp. 176–180, 1999.
- [20] S. Odenbreit, J. Puls, B. Sedlmaier, E. Gerland, W. Fischer, and R. Haas, "Translocation of *Helicobacter pylori* cagA into gastric epithelial cells by type IV secretion," *Science*, vol. 287, no. 5457, pp. 1497–1500, 2000.
- [21] R. H. Argent, M. Kidd, R. J. Owen, R. J. Thomas, M. C. Limb, and J. C. Atherton, "Determinants and consequences of different levels of CagA phosphorylation for clinical isolates of *Helicobacter pylori*," *Gastroenterology*, vol. 127, no. 2, pp. 514–523, 2004.
- [22] T. Azuma, "Helicobacter pylori CagA protein variation associated with gastric cancer in Asia," *Journal of Gastroenterology*, vol. 39, no. 2, pp. 97–103, 2004.
- [23] M. Hatakeyama, "Helicobacter pylori CagA and gastric cancer: a paradigm for hit-and-run carcinogenesis," *Cell Host & Microbe*, vol. 15, no. 3, pp. 306–316, 2014.
- [24] K. Dadashzadeh, M. P. Peppelenbosch, and A. I. Adamu, "Helicobacter pylori pathogenicity factors related to gastric cancer," *Canadian Journal of Gastroenterology and Hepatology*, vol. 2017, Article ID 7942489, 6 pages, 2017.
- [25] T. L. Cover and S. R. Blanke, "Helicobacter pylori vacA, a paradigm for toxin multifunctionality," *Nature Reviews Microbiology*, vol. 3, no. 4, pp. 320–332, 2005.
- [26] S. L. Palframan, T. Kwok, and K. Gabriel, "Vacuolating cytotoxin A (VacA), a key toxin for *Helicobacter pylori* pathogenesis," *Frontiers in Cellular and Infection Microbiology*, vol. 2, p. 92, 2012.
- [27] S. Z. Bakhti, S. Latifi-Navid, S. Mohammadi et al., "Relevance of *Helicobacter pylori* vacA 3'-end region polymorphism to gastric cancer," *Helicobacter*, vol. 21, no. 4, pp. 305–316, 2016.
- [28] E. T. Kabamba, V. P. Tuan, and Y. Yamaoka, "Genetic populations and virulence factors of *Helicobacter pylori*," *Infection, Genetics and Evolution*, vol. 60, pp. 109–116, 2018.
- [29] H. Ogiwara, M. Sugimoto, T. Ohno et al., "Role of deletion located between the intermediate and middle regions of the *Helicobacter pylori* vacA gene in cases of gastroduodenal diseases," *Journal of Clinical Microbiology*, vol. 47, no. 11, pp. 3493–3500, 2009.
- [30] R. M. Peek Jr., S. A. Thompson, J. P. Donahue et al., "Adherence to gastric epithelial cells induces expression of a *Helicobacter pylori* gene, IceA, that is associated with clinical outcome," *Proceedings of the Association of American Physicians*, vol. 110, no. 6, pp. 531–544, 1998.
- [31] L. J. van Doorn, C. Figueiredo, R. Sanna et al., "Clinical relevance of the CagA, VacA, and IceA status of *Helicobacter pylori*," *Gastroenterology*, vol. 115, no. 1, pp. 58–66, 1998.
- [32] Y. Yamaoka, T. Kodama, O. Gutierrez, J. G. Kim, K. Kashima, and D. Y. Graham, "Relationship between *Helicobacter pylori* IceA, CagA, and VacA status and clinical outcome: studies in four different countries," *Journal of Clinical Microbiology*, vol. 37, no. 7, pp. 2274–2279, 1999.
- [33] S. M. Aghdam, Z. Sardari, R. Safaralizadeh et al., "Investigation of association between OipA and IceA1/IceA2 genotypes of *Helicobacter pylori* and gastric cancer in Iran," *Asian Pacific Journal of Cancer Prevention*, vol. 15, no. 19, pp. 8295–8299, 2014.
- [34] M. M. Biernat, G. Gościński, and B. Iwańczak, "Prevalence of *Helicobacter pylori* Caga, Vaca, Icea, Baba2 genotypes in Polish children and adolescents with gastroduodenal disease," *Postępy Higieny I Medycyny Doświadczalnej*, vol. 68, pp. 1015–1021, 2014.
- [35] M. Siurala, K. Varis, and M. Wiljasalo, "Studies of patients with atrophic gastritis: a 10–15-year follow-up," *Scandinavian Journal of Gastroenterology*, vol. 1, no. 1, pp. 40–48, 1966.
- [36] N. Uemura, S. Okamoto, S. Yamamoto et al., "Helicobacter pylori infection and the development of gastric cancer," *New England Journal of Medicine*, vol. 345, no. 11, pp. 784–789, 2001.
- [37] H. Ohata, S. Kitauchi, N. Yoshimura et al., "Progression of chronic atrophic gastritis associated with *Helicobacter pylori* infection increases risk of gastric cancer," *International Journal of Cancer*, vol. 109, no. 1, pp. 138–143, 2004.
- [38] N. Kim, R. Y. Park, S.-I. Cho et al., "Helicobacter pylori infection and development of gastric cancer in Korea," *Journal of Clinical Gastroenterology*, vol. 42, no. 5, pp. 448–454, 2008.
- [39] Y. H. Park and N. Kim, "Review of atrophic gastritis and intestinal metaplasia as a premalignant lesion of gastric cancer," *Journal of Cancer Prevention*, vol. 20, no. 1, pp. 25–40, 2015.
- [40] T. Tongtawee, C. Dechsukhum, W. Leeanansaksiri et al., "Improved detection of *Helicobacter pylori* infection and premalignant gastric mucosa using "site specific biopsy": a randomized control clinical trial," *Asian Pacific Journal of Cancer Prevention*, vol. 16, no. 12, pp. 8487–8490, 2015.
- [41] World Medical Association, "World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects," *JAMA*, vol. 310, no. 20, pp. 2191–2194, 2013.
- [42] J. K. Boonjakuakul, D. R. Canfield, and J. V. Solnick, "Comparison of *Helicobacter pylori* virulence gene expression in vitro and in the rhesus macaque," *Infection and Immunity*, vol. 73, no. 8, pp. 4895–4904, 2005.
- [43] C. Schabereiter-Gurtner, A. M. Hirschl, B. Dragosics et al., "Novel real-time PCR assay for detection of *Helicobacter pylori* infection and simultaneous clarithromycin susceptibility testing of stool and biopsy specimens," *Journal of Clinical Microbiology*, vol. 42, no. 10, pp. 4512–4518, 2004.
- [44] J. T. Loh, C. L. Shaffer, M. B. Piazuelo et al., "Analysis of cagA in *Helicobacter pylori* strains from Colombian populations with contrasting gastric cancer risk reveals a biomarker for disease severity," *Cancer Epidemiology Biomarkers & Prevention*, vol. 20, no. 10, pp. 2237–2249, 2011.
- [45] J. K. Boonjakuakul, M. Syvanen, A. Suryaprasad, C. L. Bowlus, and J. V. Solnick, "Transcription profile of *Helicobacter pylori* in the human stomach reflects its physiology in vivo," *The Journal of Infectious Diseases*, vol. 190, no. 5, pp. 946–956, 2004.
- [46] T. Essawi, W. Hammoudeh, I. Sabri, W. Sweidan, and M. A. Farraj, "Determination of *Helicobacter pylori* virulence genes in gastric biopsies by PCR," *ISRN Gastroenterol*, vol. 2013, Article ID 606258, 4 pages, 2013.
- [47] F. Bibi, S. A. Alvi, S. A. Sawan et al., "Detection and genotyping of *Helicobacter pylori* among gastric ulcer and cancer patients from Saudi Arabia," *Pakistan Journal of Medical Sciences*, vol. 33, no. 2, pp. 320–324, 2017.

- [48] M. Sohrabi, R. Khashei, M. Alizadeh et al., "Low rate of babA2 genotype among Iranian *Helicobacter pylori* clinical isolates," *Journal of Clinical and Diagnostic Research*, vol. 11, no. 7, pp. DC32–DC36, 2017.
- [49] T. Tongtawe, W. Wattanawongdon, and T. Simawaranon, "Effects of periodontal therapy on eradication and recurrence of *Helicobacter pylori* infection after successful treatment," *Journal of International Medical Research*, vol. 47, no. 2, pp. 875–883, 2019.
- [50] S. Tsuji, N. Kawai, M. Tsujii, S. Kawano, and M. Hori, "Review article: inflammation-related promotion of gastrointestinal carcinogenesis—a perigenetic pathway," *Alimentary Pharmacology and Therapeutics*, vol. 18, no. s1, pp. 82–89, 2003.
- [51] M. Suganuma, K. Yamaguchi, Y. Ono et al., "TNF- $\alpha$ -inducing protein, a carcinogenic factor secreted from *H. pylori*, enters gastric cancer cells," *International Journal of Cancer*, vol. 123, no. 1, pp. 117–122, 2008.
- [52] L. Salimzadeh, N. Bagheri, B. Zamanzad et al., "Frequency of virulence factors in *Helicobacter pylori*-infected patients with gastritis," *Microbial Pathogenesis*, vol. 80, pp. 67–72, 2015.
- [53] A. T. Franco, E. Johnston, U. Krishna et al., "Regulation of gastric carcinogenesis by *Helicobacter pylori* virulence factors," *Cancer Research*, vol. 68, no. 2, pp. 379–387, 2008.
- [54] O. Teymournejad, A. M. Mobarez, Z. M. Hassan, and A. Talebi Bezmin Abadi, "Binding of the *Helicobacter pylori* OipA causes apoptosis of host cells via modulation of bax/bcl-2 levels," *Scientific Reports*, vol. 7, no. 1, p. 8036, 2017.
- [55] L. Demirturk, A. M. Ozel, Y. Yazgan et al., "CagA status in dyspeptic patients with and without peptic ulcer disease in Turkey: association with histopathologic findings," *Helicobacter*, vol. 6, no. 2, pp. 163–168, 2001.
- [56] M. Saruc, M. A. Demir, N. Kucukmetin, A. R. Kandiloglu, U. S. Akarca, and H. Yuceyar, "Histological and clinical predictive value of determination of tissue cagA status by PCR in *Helicobacter pylori* infected patients; results of the large population based study in western Turkey," *Hepatogastroenterology*, vol. 49, no. 45, pp. 878–881, 2002.
- [57] S. Kato, N. Matsukura, K. Tsukada et al., "*Helicobacter pylori* infection-negative gastric cancer in Japanese hospital patients: incidence and pathological characteristics," *Cancer Science*, vol. 98, no. 6, pp. 790–794, 2007.