STUDY ON THE PRESENCE OF *OIPA* AND *DUPA* GENES *OF HELICOBACTER PYLORI* IN GASTRIC CANCER PATIENTS

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Summary

Objectives: To determine the percentage of *Helicobacter pylori* (*H. pylori*) carrying the outer inflammatory protein A *(oipA)*, duodenal ulcer promotion *(dupA)* genes, and their relationship with endoscopic imaging, histopathology, and the risk of gastric cancer (GC). ***Subjects and methods:*** A cross-sectional study on 89 GC patients, compared with 90 patients with chronic gastritis (CG) with *H. pylori* infection, diagnosis based on histopathological results at Military Hospital 103 and Central Military Hospital 108 from August 2019 to May 2022. Determination of *oipA*, *dupA* genes of *H. pylori* by realtime-PCR technique on biopsy tissue samples of the gastric mucosa. ***Results:*** The percentage of *H. pylori* carrying the *oipA* and *dupA* genes was 30.2% and 10.1%, respectively. The relationship between bacterial infection harboring these 2 genes and endoscopic images and histopathology of GC has not been recorded. There was no difference in the prevalence of *H. pylori* carrying the *oipA* and *dupA* genes infection between the GC and CG groups ($p > 0.05$). ***Conclusion:*** The infection of *H. pylori* with *oipA* and *dupA* genes positive may not increase GC risk in Vietnamese.

* *Keywords: Helicobacter pylori; oipA, dupA; Gastric cancer (GC); Chronic gastritis (CG).*

INTRODUCTION

The *oipA* gene of *H. pylori* was first discovered in 2000 and encoded a protein of the bacterial outer membrane protein family; infection with *oipA*-positive *H. pylori* increases the risk of gastro-duodenal ulcers and GC [1].

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Date received: 27/01/2023
Date accepted: 08/02/2023
http://doi.org/10.56535/jmpm.v48i2.279

The *dupA* gene is located in the plasticity region of the *H. pylori* genome which encodes a protein that is involved in duodenal ulcer (DU) promotion [2]. Initially, Lu et al. (2005) in Japan discovered that *dupA* was linked to an increased risk of DU and protected against GC [2]. Since then, there have been several investigations on the function of *dupA*, some of which have supported the findings of Lu's study and others of which, including those from Japan, have not [3]. In terms of pathogenesis, *oipA* and *dupA* genes are both associated with increasing IL-8 concentration in the gastric mucosa [4]. Thus, they can be present together and interact synergistically during the pathogenesis.

In Vietnam, there are limited studies on these genes, especially in GC patients, so we conducted this study with the following objectives: *Determining the proportion of H. pylori carrying oipA, dupA genes and the relationship between them with endoscopic imaging, histopathology, and gastric cancer risk.*

SUBJECTS AND METHODS

1. Subjects

The study was conducted on 89 GC, and 90 CG patients infected *H. pylori* examined at Military Hospital 103 and 108 Central Military Hospital from August 2019 to May 2022.

* *Recruited criteria*: Patients presented with upper gastrointestinal symptoms, underwent esophago-gastroduodenoscopy, and histopathology from gastric mucosal biopsies is used to diagnose GC and CG. Patients with *H. pylori* infection are identified when both the rapid urease test as well as the PCR on tissue biopsy are positive.

* *Excruited criteria*: GC has been treated (chemotherapy, radiation therapy, surgery), GC secondary to metastasis, GC associated with other organ cancer. Patients used antibiotics, bismuth-containing drugs, NSAIDs within 4 weeks, and H2-receptor antagonists or PPIs within 2 weeks prior to the study. The DNA sample extracted from the biopsy was of poor quality and the patient refused to participate in the study.

2. Methods

* *Study design*: A cross-sectional study, comparison between groups.

* *Study process*:

Patients recruited in the study were clinically examined, performed appropriate tests, and then underwent esophago-gastroduodenal endoscopy, gastric mucosal tissue biopsy according to the Sydney 1994 consensus to evaluate histopathology and *H. pylori* test, GC patients had 3 additional biopsied samples in the tumor. Evaluation of the macroscopic image of GC according to Borrmann's classification, including

4 types: Mass (type 1), ulcerative (type 2), infiltrating ulcerative (type 3), and diffuse infiltrative (type 4); histopathological assessment according to Lauren's classification (1965), including intestinal type and diffuse type.

Determination of the presence of *oipA*, *dupA* genes: Biopsies samples are used to determine *oipA* and *dupA* were fixed in transport medium, immediately refrigerated at 4^{0}C for 24 hours, then transferred to -80^{0}C until processed. *H. pylori* DNA from biopsies was extracted using Monarch® Genomic DNA Purification Kit (NEB, USA), then purified, and *oipA*, *dupA* status was determined by realtime-PCR. The results were analyzed at the Department of Molecular Biology - Central Military Hospital 108.

* *Statistical analysis*: The data were analyzed using STATA 14.2. Calculating mean, standard deviation with quantitative variables and percentage, analyzing the difference by Chi-square test or Fisher's exact test qualitative variables, the p-value < 0.05 are accepted as statistically significant differences.

RESULTS

* *General characteristics:*

Table 1: Characteristics of age, sex, endoscopic and histopathologic images of GC (n = 89).

Characteristics		Number (n)	Percentage (%)
Age group	≤ 40	3	3.4
	41 - 50	8	9.0
	51 - 60	23	25.8
	61 - 70	24	27.0
	> 70	31	34.8
Mean age	64.9 ± 12.2		
Sex	Female	24	26.9
	Male	65	73.1
Macroscopic imaging (Borrmann's classification)	Type I	5	5.6
	Type II	23	25.9
	Type III	61	68.5
	Type IV	0	0
Microscopic imaging (Lauren's classification)	Intestinal type	39	43.8
	Diffuse type	50	56.2

The mean age of the GC group was 64.9 ± 12.2. The proportion of GC increases with age. The group over 70 years old accounts for the highest rate (34.8%).

There was no case of early GC (type 0), the rate of Borrmann type III accounted for the majority (68.5%), and no cases of type IV were recorded.

The percentage of diffuse type predominates intestinal type (56.2% versus 43.8%).

** Percentage of H. pylori carrying oipA and dupA genes in GC group:*

Table 2: Proportion of *H. pylori* carrying *oipA* and *dupA* genes in GC group (n = 89).

Genes presentation		Number (n)	Percentage (%)
oipA	Negative	64	71.9
	Positive	25	28.1
dupA	Negative	81	91.0
	Positive	8	9.0

In GC patients, the rate of *H. pylori* carrying the *oipA* and *dupA* genes was 28.1% and 9.0%, respectively.

** The relationship between oipA and dupA genes status of H. pylori with endoscopic images and histopathology in GC patients:*

Table 3: The relationship between *oipA* and *dupA* genes status with macroscopic imaging in GC group.

Macroscopic imaging (Borrmann's classification)	*oipA*		*dupA*	
	Negative (n, %)	Positive (n, %)	Negative (n, %)	Positive (n, %)
Type I (n = 5)	5 (100)	0 (0)	4 (80.0)	1 (20.0)
Type II (n = 23)	14 (60.9)	9 (39.1)	20 (86.9)	3 (13.1)
Type III (n = 61)	45 (73.8)	16 (26.2)	57 (93.4)	4 (6.6)
p	> 0.05			

There was no significant difference in the prevalence of *H. pylori* infection carrying the *oipA* and *dupA* genes according to Borrmann's classification (chi-square test, p > 0.05).

Table 4: The relationship between *oipA* and *dupA* genes status with microscopic imaging in GC group.

Microscopic imaging (Lauren's classification)	*oipA*		*dupA*	
	Negative (n, %)	Positive (n, %)	Negative (n, %)	Positive (n, %)
Intestinal type (n =39)	32 (82.1)	7 (17.9)	34 (87.2)	5 (12.8)
Diffuse type (n = 50)	32 (64.0)	18 (36.0)	47 (94.0)	3 (6.0)
p	> 0.05			

There was no significant difference in the prevalence of *H. pylori* to carry the *oipA* and *dupA* genes according to Lauren's classification (chi-square test, p > 0.05).

** The risk of GC when infected with H. pylori carrying oipA and dupA genes*

In order to estimate the risk of GC when infected with *H. pylori* strains carrying the *oipA* and *dupA* genes, we compared the percentage of the status of 2 genes between the GC and CG groups. The findings are shown in Table 5.

Table 5. Comparison of the proportion of *H. pylori* carrying the *oipA* and *dupA* genes between the GC and CGgroup.

Gene status		Chronic gastritis		GC		Total (n, %)	p
		n	%	n	%		
oipA	Negative	61	67.8	64	71.9	125/179 (69.8)	> 0.05
	Positive	29	32.2	25	28.1	54/179 (30.2)	
dupA	Negative	80	88.9	81	91.0	161/179 (89.9)	
	Positive	10	11.1	8	9.0	18/179 (10.1)	
Combination $oipA^+/dupA^+$	Absence	87	96.7	84	94.4	171/179 (95.5)	> 0.05*
	Presence	3	3.3	5	5.6	8/179 (4.5)	

Note: *: Fisher exact test.

There was no difference in the prevalence of *H. pylori* carrying the *oipA*, *dupA* gene (chi-square test, p > 0.05) as well as a combination of *oipA*/*dupA* (Fisher exact test, p > 0.05) between GC and CG group.

DISCUSSION

There were many studies on the role of *oipA* in *H. pylori*-associated gastroduodenal diseases, and there are two types of study designs: Studies based on the presence/absence of *oipA* or studies based on the *oipA* functional status (functional/non-functional *oipA*). However, the results of these studies are inconsistent. The results of this study showed that the rate of *oipA* positive was 30.2%, and the distribution was relatively even between the two groups (CG was 32.2%; GC was 28.1%). The difference is not statistically significant ($p > 0.05$). Regarding the association between *H. pylori* infection carrying *oipA* genotype with age, sex, endoscopic and histopathological characteristics in the GC group, the results showed that there was no significant difference. Evidence that supports *oipA* status as a risk factor for GC includes the ability to attach to gastric epithelial cells, induce inflammation through induction of IL-8 secretion, apoptosis, and cytotoxicity against gastric epithelial cells in the experiment [1]. A study in Taiwan revealed that the frequency of *oipA* in GC patients was significantly higher than in peptic ulcer patients and non-GC patients with OR of 7.38 and 8.53, respectively [5]. However, the study by Feili O (2021) in Iran reported that the rate of *oipA* gene positive in the GC group (47.4%) was lower than that of the non-atrophic CG group (70.3%) and concluded the *oipA* genotype reduces the risk of GC ($OR = 0.379$; $p < 0.001$) [6]. In fact, it is difficult to explain this difference, possibly due to the difference in subjects, geographic location, socioeconomic status, and host and environmental factors. Another remarkable feature to explain the difference between studies is that most studies to determine *oipA* gene status are based on sequencing to detect the number of repeats of C-T pair nucleotide by PCR. However, the analysis and identification of results based on PCR method become complicated because of the great genetic diversity of *H. pylori*, leading to the possibility of underestimation of the status of gene function. However, this finding is consistent with the study of Nguyen Lam Tung (2010) when confirming the percentage of functional *oipA* found in 100% *H. pylori* strains [7], so it can be speculated that *oipA*-positive status may not be a risk factor for *H. pylori*-associated gastrointestinal diseases in Vietnam.

The *dupA* gene was initially responsible for promoting duodenal ulcers [2]. However, some studies did not recognize this finding [3]. The current study results indicate that the

rate of *dupA* gene positive was 10.1%, of which the CG group was 11.1% and the GC group was 9.0%. The difference is not statistically significant ($p > 0.05$). In terms of the relationship between *H. pylori* infection carrying *dupA* positive with age, sex, endoscopy, and histopathology of the GC group, the results showed there was no statistically significant difference, which is consistent with several studies from Brazil and Iran [8]. The discrepancy between studies, in addition to differences in *dupA* prevalence by region and characteristics of study subjects. Gomez et al. reported that 16.3% of *dupA* sequences from Brazil had a frameshift mutation, generating an early termination codon that can significantly affect protein expression [9]. This finding raises questions about the presence of similar frameshift mutations elsewhere in the *dupA* sequence that cannot be detected by PCR method. Therefore, an immunoblot technique with anti-dupA antibodies is needed to elucidate the true relationship between *dupA* and clinical outcomes.

In terms of *H. pylori* harboring a combination of *oipA*/*dupA*, we found that this rate in the GC was higher than that of the CG group (5.6% versus 3.3%), but the difference was not statistically significant. This result is different from the study of Dadashzadeh (2017) in Iran that the combination of *oipA/dupA* reduced the risk of GC ($p < 0.05$) [10], the disparity between the two studies was due to the fact that in the study of Dadashzadeh, negative *dupA* status was associated with GC development [10].

CONCLUSION

The rate of *oipA* and *dupA* of *H. pylori* was 30.2% and 10.1%, respectively. There was no association between the status of *H. pylori* carrying *oipA, dupA* positive with endoscopic and histopathologic images in GC group. There was no significant difference in the prevalence of *H. pylori* carrying the *oipA* and *dupA* positive between the GC and CG groups ($p > 0.05$).

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