RESEARCH ARTICLE

Editorial Process: Submission:11/13/2022 Acceptance:03/10/2023

Relationship between *Helicobacter Pylori* Infection and the Risk of Esophageal Cancer in Thailand

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Abstract

Objective: Esophageal cancer (EC) is a multifactorial disease and a leading cause of mortality. Epidemiological and molecular studies have provided evidence that Helicobacter pylori (*H. pylori*) infection is an important cause of gastric carcinogenesis and thus, may be related to EC. However, esophagus *H. pylori* infection in Thai patients with newly diagnosed EC has not been reported. Moreover, the evidence of the association with *H. pylori* to EC is controversial. This study investigated the possible association between *H. pylori* infection with a virulence gene and EC in Thailand. **Methods:** A case-control study was conducted that involved 105 newly diagnosed EC patients and 108 healthy controls. The prevalence of *H. pylori* infection detected in formalin-fixed, paraffin-embedded EC tissue in esophageal biopsy specimens from the subjects was measured using real-time PCR. All the data were collected in face to face interviews using a structured questionnaire. Multivariable unconditional logistic regression was used to calculate and analyses the odds ratios (ORs) of the data. **Results:** A significant association was found between *H. pylori* infection and EC ($p < 0.001$, 95% CI:3.11–10.48). *H. pylori*-positive subjects had a 2.76 times higher risk of developing ESCC. Moreover, the *H. pylori*-positive subjects who were *CagA*-positive had slightly higher ORs and statistically significant risk factors. **Conclusions:** *H. pylori* infection was found to be associated with a risk of EC in Thailand, and among the *H. pylori*-positive subjects who were *CagA*-positive had a higher risk factor of ESCC but not of EAC.

Keywords: Helicobacter pylori- esophageal cancer- risk factor- case-control study

Asian Pac J Cancer Prev, **24 (3)**, 1073-1080

Introduction

Esophageal cancer (EC) represents a major problem globally. It is one of the most frequently occurring malignancies in the world, and its incidence and mortality are increasing (Ferlay et al., 2019). There are two major histological subtypes of EC: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). ESCC and EAC have different demographic and geographic models (Gao et al., 2019). In Thailand, EC is a chronic disease, a serious health problem and the eighth leading cause of death. Its overall estimated age standardized incidence rates among men and women in 2015 are 4.8 and 1.4 cases per 100,000, respectively. Moreover, the number of new cases increase every year (Imsamran W et al., 2015). Many studies have confirmed the role of tobacco smoking, alcohol drinking and poor oral hygiene in the etiology of EC (Chen et al., 2017; Geßner et al., 2021; Jayalekshmi et al., 2021). Some studies have found, however, that Helicobacter pylori (*H. pylori*), an infectious agent that is known to be an important risk factor of gastric cancer and as associated with other types of cancer locally (Boubrik et al., 2022; Carlosama-Rosero et al., 2022), can also contribute etiologically to the occurrence of EC. Biological specimens had been collected to explore this possible link with EC. However, the association between *H. pylori* infection and EC is still being debated (Leon et al., 2019). *H. pylori* is a microaerophilic helical-shaped Gram-negative bacterium in the upper digestive tract that has been identified as the major causative agent of various benign and malignant gastrointestinal tract diseases (Handa et al., 2011). The World Health Organization and the International Agency for Research on Cancer consensus group have classified *H. pylori* as a class I human carcinogen because of its relationship to gastric carcinoma (El-Shenawy A et al., 2017). More and more studies are reporting about the pathology, immunology and virulence of *H. pylori*. It has been previously established, however, that *H. pylori* infection varies geographically, and developing nations carry the higher burdens. Recently, epidemiologic studies have presented the positive correlation between

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H. pylori infection and some diseases, but that only 20% of *H. pylori*-infected patients develop a serious disease (Sonnenberg, 2022). The extent and the severity of these associated diseases depend on several factors, such as the bacterial virulence factors and the host factors such as the immune system and environmental determinants (Amieva et al., 2016; Yamaoka et al., 2014). *H. pylori* has a number of virulence factors that play a role in its pathogenesis and that influence its colonization, invasion and disease severity (Maleki Kakelar et al., 2019; Raghwan et al., 2014). Previous studies demonstrated that the virulence genes of *H. pylori* play an important role in its pathogenicity (Baj et al., 2020). The most widely studied *H. pylori* virulence factor is the cytotoxin-associated gene A (*CagA*) antigen, a 96–138-kDa protein. The *CagA* gene was found in the genomic region called the Cag pathogenicity island (PAI), and it is considered a marker of an enhanced virulence gene. *H. pylori* strains that are *CagA*-positive are associated with severe inflammation and increased risk of ulcers and cancer in humans (Noto et al., 2012; Palrasu et al., 2020; Yamaoka et al., 2014). Various studies have been conducted to demonstrate the relationship between virulence genes of *H. pylori* and the severity of gastrointestinal diseases, and most of those studies investigated *CagA* genes (Abu-Taleb et al., 2018). Several epidemiological studies have examined the association between *H. pylori* infection, including infection with the more virulent *CagA*-positive strains, and the risk of EC (Anderson et al., 2008). However, such studies have reported inconclusive results, and some of them even found a reverse relationship between *H. pylori* and ESCC after performing a serological test or ELISA (Xie et al., 2013). Similar studies have presented some evidence, following polymerase chain reaction (PCR), that *CagA*-positive *H. pylori* infection is associated with the risk of EC or gastric cancer (Glocker et al., 1998; Krashias et al., 2013; Li et al., 2014). In Thailand, the prevalence of *H. pylori* virulence gene infection in the upper aerodigestive tract (UADT), which includes the esophagus, is unknown in the general population but only in patients with EC undergoing upper endoscopy, and no study has simultaneously examined the relationship between the *H. pylori CagA* virulence gene and EC patients. Therefore, the main objective of this study is to investigate the association between *H. pylori CagA* gene infection and EC in patients in a hospital through a case-control study to inform the planning of a large-scale etiological study of UADT cancer in Thailand.

Materials and Methods

Study design and sample collection

This study is a hospital-based case-control study that involved 105 patients with EC and 108 healthy controls recruited from Srinagaring Hospital in Khon Kaen province, northeast Thailand, from 2007 to 2017. Case subjects were defined as patients who had a histopathologically confirmed diagnosis of EC. Control subjects were patients with a diagnosis of non-cancer condition. They were selected from patients undergoing routine endoscopy for investigation of presumed nonmalignant conditions such as gastroesophageal reflux disease. All the patients were admitted at the same time and in the same ward as cancer cases. Pathological physicians diagnosed the inclusion criteria in all the cases. The data on risk factors were collected by a trained interviewer using a standardized questionnaire developed by researchers. EC specialists confirmed the validity of the content of the questionnaire. All the subjects were asked to answer, face to face, another questionnaire on their demographic characteristics and the history of their illness. Tissue samples were collected from all the subjects. The specimen tissue was separated into two groups: EC cases and Control subjects. ESCC and EAC tissues were included in the EC cases group, and normal esophageal tissues were put in the Control subjects group. All these 213 retrospective formalin-fixed, paraffin-embedded (FFPE) tissue samples, which included 105 EC and 108 normal tissues, were retrieved from paraffin blocks stored at Khon Kean University (Department of Pathology, Faculty of Medicine) from 2007 to 2017. They were initially diagnosed by a specialist according to the International Classification of Diseases for Oncology Third Edition and confirmed via electronic gastroscopy and histopathology reports. This study was approved by the Khon Kaen University Ethics Committee in Human Research (reference no. HE621269).

DNA extraction

The total genomic DNA were extracted from the FFPE esophagus tissue using the commercially available DNeasy blood and tissue kits (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The DNA quality and concentration were quantified with a Nano Drop ND-1000 spectrophotometer (Thermo Scientific). All the DNA samples were stored at -20°C for further analyses.

H. pylori detection via SYBR Green based real-time PCR assay

After the DNA sample extraction, PCR was performed to detect *H. pylori* using specific primers, target genes, amplicon sizes, sequences and primer names. All the primers used in this study are presented in Supplementary Table 1. Real-time PCR assays were performed on an Applied Biosystems 7,500 flats system (Applied Biosystems, USA) using the SYBR Green PCR kit (Fermentous, Germany) to confirm the presence of *H. pylori CagA*-positive DNA samples. For PCR amplification, 1–2 µg of DNA samples was added to a PCR mixture that contained 12.5 µl of 2x SYBR Green mastermix, 0.3 µM each of the forward and reverse primers, and sterile water to achieve a total volume of 25 µl. PCR amplification was performed under the following conditions: initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing for 30 s, primer extension at 72°C for 30 s and final extension at 72°C for 5 min. The data were analysed using QuantstudioTM 6 Flex Real-Time PCR systems. The *H. pylori* strain was detected using specific primers that targeted 16S rRNA and ureA genes. The *CagA*-positive status was determined from *H. pylori*-positive samples via PCR using their respective primers, as described in

Supplementary Table 1. Both a positive control (DNA extracted from reference stains, the 16S RNA genes of which were sequenced) and a negative control (molecular distilled water) were used in each run of the PCR assay.

Statistical analysis

The statistical analysis was performed with STATA software, version 13.0. The proportions of the specimens that tested positive for *H. pylori* (overall, if either the ureA genes or the 16S rRNA genes were amplified) were expressed as percentages. Among the *H. pylori*-positive specimens, the proportion that tested positive for the virulence gene *CagA* were also calculated. Bivariate analysis using simple logistic regression was performed to investigate the association between the independent factors and EC, without controlling for variable confounding. Crude odds ratios (OR crude) and their 95% confidence intervals (95% CI) were determined. Multivariable unconditional logistic regression was used to compute adjusted odds ratios (OR_{adj}) and their 95% CI to investigate the association between *H. pylori* infection, *CagA* gene infection and EC while controlling for the effects of confounding variables. All the test statistics were two-sided, and a p-value of ≤ .05 was considered statistically significant.

Results

Demographic characteristics

The characteristics of the 105 patients with EC and the 108 normal controls are described in Table 1. The mean age (± SD) at diagnosis was 60.4 years (± 8.9), and 63 (60%) of the included samples were from male patients. For the demographics of the subjects' lifestyle habits, drinking, smoking and daily proton pump inhibitor (PPI) use were more prevalent among the EC cases than the controls. The frequency of a personal history of gastroesophageal reflux disease (GERD) differed between the cases and the controls (p-value = ≤ .05). There was a higher prevalence of *H. pylori*-positive specimens (79%) and *CagA*-positive specimens (24%) among the EC cases than among the controls (38% and 9.3%, respectively).

Associations of esophageal cancer (EC) and H. pylori infection

Table 2 presents the factors associated with EC and the main outcomes of the multivariable analysis. The factors found to be significantly associated with EC are alcohol consumption, smoking and a personal history of GERD. There was a statistically significant dose-response relationship (p-value < .05). We also found that among the sample genes (ureA and 16S rRNA), 123 (57%) were *H. pylori*-positive. These included 83 (79%) among the EC cases and 40 (38%) among the controls. Infection

Table 1. Characteristics of the Esophageal Cancer Cases and Controls

Characteristics	Esophageal cancer N = 105 (%)		Controls N = 108 (%)		p-value
Gender					
Female	42	40%	57	52.80%	0.074
Male	63	60%	51	47.10%	
Age (mean ± SD)	60.4 (8.9)		60.1 (9.2)		0.012
Body mass index or BMI (kg/m^2) (mean ± SD)	24.1 ± 3.62		23.4 ± 3.46		0.681
< 23.00	49	46.70%	53	51.40%	
≥ 23.00	56	53.30%	55	48.60%	
Drinking status					
Non-drinker	28	26.70%	66	61.10%	< 0.001
Drinker	77	73.30%	42	38.90%	
Smoking status					
Non-smoker	32	30.50%	79	73.20%	< 0.001
Smoker	73	69.50%	26	26.80%	
Personal history of gastroesophageal reflux					
Disease (GERD)					
No	25	23.80%	66	61.10%	<0.001
Yes	80	76.20%	42	38.90%	
Daily proton pump inhibitor (PPI) use					
≥ 1 year before interview					
No	33	31.40%	56	51.80%	0.003
Yes	72	68.60%	52	48.20%	
H. pylori infection					
Negative	20	20.90%	68	62.00%	0.001
Positive	83	79.10%	40	38.00%	

Table 2. Crude and adjusted Odds Ratios for the Associations between *H. pylori*, *CagA* Genes and Esophageal Cancer

Variables	Control n = 108	Esophageal cancer n = 105	ORC (95% CI)†	OR_{adj}(95% CI)‡	p-value
Drinking status					
Non-drinker	66 (61.1%)	28 (26.7%)	1.00 (reference)	1.00 (reference)	0.004
Drinker	42 (38.9%)	77 (73.3%)	3.3 (2.42–7.71)	2.8 (1.78–6.82)	
Smoking status					
Non-smoker	79 (73.2%)	32 (30.5%)	1.00 (reference)	1.00 (reference)	< 0.001
Smoker	26 (26.8%)	73 (69.5%)	2.2 (3.42–11.26)	2.4 (3.68–10.12)	
Personal history of gastroesophageal reflux disease (GERD)					
No	66 (61.1%)	25 (23.8%)	1.00 (reference)	1.00 (reference)	0.02
Yes	42 (38.9%)	80 (76.2%)	2.0 (2.71–9.12)	2.16 (1.15–8.82)	
Daily proton pump inhibitor (PPI) use ≥ 1 year before interview					0.083
No	56 (51.8%)	33 (31.4%)	1.00 (reference)	1.00 (reference)	
Yes	52 (48.2%)	72 (68.6%)	0.4 (0.48–4.11)	0.9 (0.91–4.08)	
H. pylori infection					< 0.001
Negative	68 (62.0%)	20 (20.9%)	1.00 (reference)	1.00 (reference)	
Positive	40 (38.0%)	83 (79.1%)	4.4 (3.48–11.82)	3.8 (3.11–10.48)	
CagA gene					
Negative	98 (90.7%)	79 (75.3%)	1.00 (reference)	1.00 (reference)	0.034
Positive	10 (9.3%)	26 (24.7%)	3.2 (1.46–7.08)	2.9 (1.08–8.04)	

*Adjusted for gender age and BMI; †ORc, crude odds ratio; ‡ORadj, adjusted odds ratio; 95% CI, 95% confidence interval; p-values obtained using unconditional logistic

with *H. pylori* increased the odds of developing EC by 33.2% (Odd_{adj}, 3.8; 95% CI, 3.11–10.48; p-value < 0.001). In 24.7% of the EC cases, *CagA*-positive genes were observed, and *CagA* was found to be significantly associated with EC (p-value = 0.034).

Association between H. pylori, CagA virulence genes and EC

As shown in Table 3, the adjusted regression analysis showed that the *H. pylori*-positive subjects had a 2.76 (95% CI, 2.55–12.19) times higher risk of developing ESCC compared to the risk of the *H. pylori*-negative subjects. The *CagA*-positive subjects had a 1.17 (95% CI, 1.52–2.63) times risk. Moreover, a *CagA*-positive gene among the *H. pylori*-positive subjects slightly increased their odds ratios and significantly increased their risk (Odd_{adj}, 1.90; 95% CI, 1.76–4.77) of developing ESCC compared with the *H. pylori*-positive but *CagA*-

Table 3. Associations between *H. pylori* Virulence Factors and Esophageal Cancer

Logistic regression model	Esophageal cancer			
No. of cases/controls	Esophageal squamous cell carcinoma (ESCC) 77/108		Esophageal adenocarcinoma(EAC) 28/108	
	OR (95% CI)	p-value	OR (95% CI)	p-value
H. pylori-positive				
Adjusted	2.76 (2.55–12.19)	< 0.001	0.67 (0.25–1.76)	0.421
Unadjusted	3.28 (3.16–9.78)	< 0.001	0.13 (0.64–3.15)	0.454
H. pylori-negative	1.00 (reference)		1.00 (reference)	
CagA-positive				
Adjusted	1.17 (1.52–2.63)	0.018	0.72 (0.05–7.03)	0.318
Unadjusted	1.52 (1.74–3.18)	0.022	0.39 (0.41–8.17)	0.284
CagA-negative	1.00 (reference)		1.00 (reference)	
CagA among *H. pylori*-positive subjects				
CagA-positive and *H. pylori*-positive subjects				
Adjusted	1.90 (1.76–4.77)	0.016	0.44 (0.82–7.26)	0.11
Unadjusted	2.52 (1.08–5.68)	0.031	0.39 (0.16–8.27)	0.215
CagA-negative and *H. pylori*-positive subjects	1.00 (reference)		1.00 (reference)	

*Adjusted for gender; age; alcohol drinking; smoking; GERD; PPI use, proton pump inhibitor; OR_{adj}, adjusted odds ratio; 95% CI, 95% confidence interval; p-values obtained using unconditional logistic regression.

negative subjects. No significant association was found between EAC and *H. pylori* positivity (Odd_{adj}, 0.67; 95% CI, 0.25–1.76), *CagA* positivity (Odd_{adj}, 0.72; 95% CI, 0.05–7.03) or *CagA* positivity among *H. pylori*-positive subjects (Odd_{adj}, 0.44; 95% CI, 0.82–7.26).

Discussion

To our knowledge, current data show that smoking, drinking alcohol, and a personal history of GERD and *H. pylori* infection are all associated with an increased risk of EC and are the major etiology of EC in the Thai population. Interestingly, these important data also found an association between *H. pylori* infection and an increased risk of ESCC. Several studies have focused on the diversity of *H. pylori* infection and its virulence genes in different countries (Ofori et al., 2019; Subsomwong et al., 2017). Whereas a previous systematic review has reported that the association of *H. pylori* infection with EC was controversial, because almost all the studies have confirmed of *H. pylori* infection using a serological test, the test might have involved overlapping cases of esophageal and gastric infections (Xie et al., 2013). Two studies confirmed that esophageal infection with *CagA*-positive *H. pylori*, which was detected via PCR, was associated with ESCC (Krashias et al., 2013; Li et al., 2014). However, there is little information available related to the frequency of *H. pylori CagA* infection among EC patients in Thailand, despite the fact that *CagA* is the protein necessary for NF-κB activation, which results in IL-8 secretion, neutrophil accumulation and tissue necrosis (Glocker et al., 1998) . Therefore, we conducted a case-control study to detect the *H. pylori CagA* gene DNA in the esophagus tissue of EC patients and normal controls using real-time PCR. Our study investigated the relation of the *CagA* virulence gene in *H. pylori* infection and if it is associated with the incidence of EC. In this study, we observed a high prevalence (57.7%) of *H. pylori* infection in the esophagus tissues of our 213 subjects. Similarly, several studies found from a nationwide survey in Thailand that among 1,546 patients from 17 provinces in four regions, the overall prevalence of *H. pylori* infection was 55.9% (Uchida et al., 2015), and some studies based on culture and histology immunohistochemistry found that the prevalence of *H. pylori* infection was 54.5% in North Thailand (Subsomwong et al., 2017). These findings are consistent with those of other studies (Tunruttanakul S et al., 2018). However, studies from other countries in the same region, such as Vietnam and Malaysia, have shown low *H. pylori* prevalence, epidemiologically diverse *H. pylori* infections, and different prevalence rates in different geographical locations (Fock et al., 2010). An important finding is that *H. pylori* positivity was associated with EC in Thailand. In this study that used archived FFPE tissues of EC patients diagnosed in the years 2007–2017, *H. pylori* DNA was detected in 79.1% of the tissues, which demonstrated that *H. pylori* infection was associated with a progression of EC. This result revealed that *H. pylori* infection is associated with a statistically significant increased EC risk (Odd_{adj}, 3.8; 95% CI, 3.11–10.48). These data also reported an odds ratio higher than the averages found in previous studies (Wang et al., 2006; Whiteman et al., 2010). The increased higher risk observed in northeast Thailand may reflect the predominance of other risk factors in this population. Other variables, such as a family history of cancer in a first-degree relationship and lifestyle habits such as the diet of Thai people were associated with EC, and the magnitude of these effects was strong enough to achieve statistical significance (Boonyaphiphat et al., 2002). This study also investigated a possible correlation between the presence of the major virulence factors of *H. pylori* and EC. Our study analyzed the presence of the most prevalent virulence gene of *H. pylori* in 123 EC patients who were *H. pylori*-positive. The most prevalent of the virulence genes was *CagA*, which was found in 24% of the EC patients. *CagA* is in fact one of the most studied virulence genes of *H. pylori*. This protein has a molecular size of 138 kDa and is found in the *CagA*-PAI. *H. pylori* strains with *CagA*-PAI are more pathogenic than strains without *CagA*-PAI (Fischer, 2011). This study showed that *CagA*-positive *H. pylori* strains were significantly associated with EC (p-value < .001). More importantly, the results showed that EC patients with *CagA*-positive *H. pylori* strains had a significantly higher odds ratio and a statistically significant risk of developing ESCC (OR_{adj}, 1.9; 95% CI, 1.76–4.77). However, we found no significant association between *H. pylori* or *CagA* positivity and EAC. On the contrary, *H. pylori*-*CagA* positivity tended to decrease the risk of EAC. A previous population-based study found that *CagA* positivity was associated with an increased risk of ESCC (OR_{adj}, 2.1; 95% CI, 1.1–4.0) (Ye et al., 2004). A prospective study in China reported similar results (Wang et al., 2006). The results also agree with previous reports of *CagA* positivity in more than 60% of cases in East Asian countries, including Thailand (Chomvarin et al., 2008). Several studies have indicated that *CagA*-positive *H. pylori* strains are more virulent than *CagA*-negative strains. Moreover, the differences between the *CagA* genotypes are associated with the severity of human cancer. The *CagA* toxin plays a very important role in carcinogenesis in the stomach and other organs when *CagA*-synthesising *H. pylori* is detected. A specific system of *CagA* genes encodes the synthesis of the *CagA* toxin, which demonstrates the properties of reorganizing the cytoskeleton and the cell shape. Moreover, the toxin controls the transcription and proliferation of the cell, which leads to a chronic inflammatory reaction (Hatakeyama, 2017; Wroblewski et al., 2016). Many recent studies of animal and cell culture models indicated the likely role of *CagA* and Cag-PAI, such as EPIYA, in human gastric cancer associated with *H. pylori* infection (Sukri et al., 2020). This study is one of the very few studies that documented *H. pylori* testing of esophageal tissue specimens. *H. pylori* is a common found in the superficial mucosal layer of the stomach and also in the esophagus. There is some evidence that *H. pylori* plays a role in the progression of GERD to Barrett's esophagus to dysplasia to EC (Kountouras et al., 2012). The evidence specifically found that *H. pylori* may be involved in the pathophysiology of GERD via diverse mechanisms such as induction of mediators, cytokines and nitric oxide,

which may disturb the lower esophageal sphincter and directly injure the esophageal mucosa due to bacterial products (Kountouras et al., 2006; Zhao et al., 2020). Another study suggested that *H. pylori* infection activates NF-κB, an oxidant-sensitive transcription regulator of the inducible expression of inflammatory genes, including COX-2, which regulates gastrointestinal neoplasm cell growth and proliferation. Specifically, *H. pylori* infection promotes the expression of NF-κB and COX-2 in esophageal epithelial cells, playing a role in the inflammatory process associated with BE and esophageal oncogenesis (Polyzos et al., 2018). Besides *CagA*, another possible cause of the activation of NF-κB and IL-8 secretion is esophageal metaplasia through chronic inflammation (Glocker et al., 1998; Li et al., 2014). A study inferred that *H. pylori* infection can cause ESCC through gastric atrophy, which may promote the excessive growth of bacteria and increase the production of endogenous nitrosamine, which may lead to ESCC (Iijima et al., 2010). The different virulence factors of *H. pylori* strains are regarded as important in causing various types of EC cancer in infected individuals. *CagA* is considered the main major virulence marker of *H. pylori* infection. Therefore, the combination of the virulence gene of *H. pylori*, the host factors and the environmental factors should be investigated simultaneously. In addition, these findings again emphasize the importance of *CagA* positivity in the pathogenesis of *H. pylori* infection associated with EC in the Thai population. Regarding other factors related to EC disease, the lifestyle may affect the severity of the disease, such as the use of medicinal plants in food and the diets and habits of the different populations. Further studies are needed to explore the mechanism by which *CagA*-positive *H. pylori* is inversely associated with the risk of EAC and to confirm the positive association between *CagA*-positive *H. pylori* and ESCC. Especially, the Cag-PAI rearrangement and the *CagA* polymorphism should be elucidated in different geographical regions for more molecular epidemiological data and pathogenesis. Moreover, both co-infection with *H. pylori* and Campylobacter spp and a single bacterial infection were also significantly associated with EC. This may indicate that these two bacteria are involved in the pathogenesis of EC. This study also had several limitations. A main limitation concerns the retrospective case-control design, from which a cause-effect relationship between *H. pylori* infection and EC risk was established. In this study, the recall bias of the measured risk factors might have affected our results, although efforts were made to minimize this bias. Moreover, we could not obtain enough information about the sociodemographic characteristics and the dietary and other lifestyle risk factors of the subjects. These factors could have influenced the EC outcome. Another limitation of the study was that the selected hospital controls might not have accurately reflected the exposure distributions of the source population and thus, might have affect the results.

In summary, the findings in this study give more evidence of the association of *H. pylori* infection and *CagA*-positive with other well-known risk factors such as tobacco smoking, alcohol drinking and personal history of GERD to elucidate the effect of those risk factors in the prevalence of *H. pylori* in EC. Our study showed a significant effect of *H. pylori* infection as risk of EC. Likewise, smoking status, alcohol use and GERD were associated with EC in our sample, the magnitude of these effects were strong enough to achieve statistical significance. Therefore, our results may have policy implications in the realize that population education and awareness of the results. In addition, the results in this study might have useful roles of the clinical helps to effectively diagnose and treat EC patients by understanding the trend of *H. pylori* infection in Thailand.

Author Contribution Statement

Poosari A , Nutravong T , Namwat W, Sa-ngiamwibool P contributed to the conception and designed the experiments. Ungareewittaya P, Poosari A, Sa-ngiamwibool P pathological data and tissue esophageal cancer (FFPE) sample. Poosari A , Nutravong T performed the experiments and reviewed statistic analyzed the data. Poosari A , Nutravong T, Boonyanugomol W wrote the initial draft of the manuscript. All of the authors contributed to the editing and approved the final version of this manuscript..

Acknowledgements

This work was financially supported by Faculty of Medicine, Khon Kaen University, Thailand (Grant no. IN62314). We also sincerely thank Department of Epidemiology and Biostatistics, Faculty of Public Health, Khon Kaen University for their contributions to data management and statistical support.

Funding Statement

This work was financially supported by Faculty of Medicine, Khon Kaen University, Thailand (Grant no. IN62314)

Ethics declarations

This present study was approved by the Khon Kaen University Ethics Committee for Human Research, based on the Declaration of Helsinki and the ICH Good Clinical Practice Guidelines; reference number HE621269.

Data Availability

The data and their analysis are available.

Conflict of interest

There was no conflict of interest.

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