CagA-Positive *Helicobacter Pylori* and the Gastroduodenal Pathology

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Abstract

In this study, an association between the infection of *Helicobacter pylori* strain producing the cytotoxin-associated antigen (CagA) and the gastroduodenal pathology was studied in a group of Thai patients with clinical dyspepsia. Investigation of *H. pylori* in the patients' gastric specimens and the *CagA* gene in *H. pylori*-positive samples were carried out by a DNA amplification technique. The result showed that the infection of *H. pylori* in the patients herein was not as high as those that were studied by other groups and was not significantly different among individuals with different gastric lesions ($p > 0.05$). Detection of the *CagA* gene in *H. pylori*-infected samples demonstrated that the prevalence of the CagA-positive *H. pylori* was lower than those that were reported elsewhere. This might be due to either a non-homogeneous distribution of the bacteria in the gastric samples or an unexpected irregular amplification of the *CagA* gene in some strains of *H. pylori*. Statistical analysis revealed no association between the presence of the bacterial *cagA* gene and the host gastric epithelial pathology ($p > 0.05$).

Keywords: *H. pylori*, CagA, virulence factor, gastroduodenal pathology

1. Introduction

Helicobacter pylori, a curved gram negative bacilli, is now recognized as a major cause of gastritis and peptic ulcer [1,2] and is classified as a carcinogenic factor for gastric cancer [3]. *H. pylori* well adapts to colonize in the stomach of humans (and other vertebrates) for the life span of the host. Although a large number of people around the world are reported to be infected with this organism and have gastric inflammation, most infected individuals do not develop dyspepsia and other gastroduodenal disorders [4,5]. Therefore, the diagnosis is overlooked at the early stage of the infection. The difference in bacterial virulence markers was thought to be one of the major factors responsible for this silent infection [6]. It was reported that individuals infected with *H. pylori* strains producing 128-140 kDa proteins, encoded by the cytotoxin-associated antigen (*CagA*) gene, had remarkable inflammation with a large number of neutrophils on the gastric mucosa [7]. It was also believed that prolonged inflammation may lead to gastric epithelial cell injury and damage from the reaction of reactive oxygen or nitrogen species, produced by the activated neutrophils. In addition, the resulted DNA damage in the epithelial cells via the formation of DNA adducts can lead to cell transformation and finally to cancer [8]. This hypothesis was supported by the observation that increased risks of gastritis, peptic ulcer and gastric cancer are associated with the *H. pylori* CagA-positive strains [5].

An association of the CagA marker and the pathology of the stomach is still a subject of controversy. It was speculated that polymorphisms in the *CagA* gene might be responsible for this argument. The prevalence of the CagA-positive *H. pylori* in Thai dyspeptic patients was lately reported by Pongchairerks *et. al.* [9] but the experiment was performed in Japan. To our knowledge, the DNA amplification based detection of the virulence *H. pylori* has rarely been performed in Thailand. Thus, it was the aim of our study to employ a

DNA amplification technique to detect the CagA-positive *H. pylori* directly from the gastric biopsy and to investigate its relationship to the gastroduodenal lesion.

2. Materials and methods

Specimen collection

Gastric biopsied specimens were obtained from 115 patients (whose endoscopic findings, sex and age are shown in Table 1) who came for gastroduodenal endoscopy in the Division of Surgery, Thammasat Hospital, Thailand from year 2000 to 2001.

Table 1 Endoscopic findings, sex and age of 115 dyspeptic patients

Endoscopic findings	Female/Male	Age (years)	
		Range	Mean ± SD
Normal[a]	9 / 14	18 - 65	33.8 ± 12.8
Erosion[b]	19 / 21	20 - 73	39.7 ± 16.5
Ulcer[b]	30 / 15	19 - 80	50.7 ± 18.6
Others[c]	5 / 2	18 - 70	42.7 ± 17.6
Total	63 / 52	18 - 80	43.0 ± 17.9

[a] Normal gastric mucosa under endoscopy
[b] Either gastric or duodenal erosion/ulcer
[c] Patients with gallstone, hemorrhagic gastritis, annual check up, cancer

All cases had clinical dyspepsia. In each patient, endoscopic diagnosis was recorded and 3-4 biopsied specimens were taken from greater curvature of the antrum close to the pyloric ring. One of the biopsied specimens was sent for *H. pylori* investigation by DNA amplification, and the remaining cimens were kept for other purposes. The use of these specimens for research was approved by the internal review board of the Faculty of Medicine, Thammasat University, Thailand.

DNA amplification by polymerase chain reaction (PCR)

H. pylori DNA was co-extracted with human DNA from the gastric biopsy by the DNA extraction kit (Qiagen, USA) according to the manufacturer's recommendation. To detect *H. pylori* in the biopsied sample, PCR was performed using a pair of primers bound specifically to *H. pylori ureC* gene: 5'-AAGCTT TTAGGGGTGTTAGGGGTTT-3' and 5'-AAG CTTACTTTCTAACACTAACGC-3' [10,11]) that was renamed the *phosphoglucosamine mutase* (*glmM*) gene, [12]. For the *H. pylori*-positive sample, the CagA virulence marker was further analysed by PCR using a pair of CagA-specific primers : 5'-AATACACCAACG CCTCCAAG-3' and 5'-TTGTTGCCGCTTTTG CTCTC–3' [13]. Primers for human pi class *glutathione-S-transferase* (*hGSTP*) gene: 5'-CTCTATGGGAAGGACCAGCAGGAG-3' and 5'-CAAGCCACCTGAGGGGTAAGG-3' [14] were added in the reaction for *H. pylori* detection (glmM PCR) as well as the primers for *glmM* gene were also added in the reaction for the CagA detection (CagA PCR) as internal control primers. Both the glmM and CagA PCR reactions were performed as described previously [13]. The PCR condition was 95°C; 40 sec, 55°C; 40 sec, 72°C; 40 sec (40 cycles). Electrophoresis of the amplified DNA was done on 1.5% agarose gel in 1X TBE buffer.

Statistical analysis

Chi-square or Fisher's exact test was used to analyse the association between type of gastric lesion and *H. pylori* infection as well as the presence of the *CagA* gene. The level of significance was set at $p < 0.05$.

3. Result

Detection of *H. pylori* infection by polymerase chain reaction in 115 patients was performed. The glmM primers that are specific for *H. pylori* detection have been used to detect the bacteria in the gastric biopsied specimens. After DNA amplification, the expected 294 bp PCR product of the *glmM* gene was observed in DNA from the *H. pylori* isolate (positive control) as well as some of the biopsied specimens (Fig.1A). The 192 bp PCR product of the *hGSTP* gene from the co-purified human gastric DNA was also present in all samples (as the internal control) indicating no interference in the DNA amplification process. No DNA band was seen when bacterial DNA was replaced by distilled water (negative control). The data in Table 2 show that the number of *H. pylori*-positive samples detected by the glmM PCR was 28.7% (33 in 115) and there was no correlation between gastroduodenal pathology and *H. pylori* infection ($p > 0.05$).

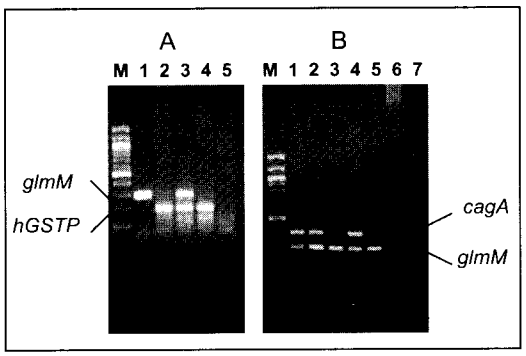

Fig. 1 DNA amplification of the biopsied specimens. A: detection of *H. pylori glmM* gene (294 bp.) and B: detection of *H. pylori CagA* gene (400 bp.). Primers for *hGSTP* (192 bp.) and *glmM* genes were also added into the PCR reactions in Figs 1A and 1B respectively as internal controls. 100 bp DNA ladder (New England Biolabs, USA) was used as a molecular size standard (M).

A: 1 = *H. pylori* isolate; 2 and 4 = biopsied specimens without *H. pylori*; 3 = a biopsied specimen with *H. pylori*; 5 = negative control
B: 1 = *H. pylori*-CagA positive isolate; 2 and 4 = biopsied specimens with *H. pylori*-CagA positive; 3 and 5 = biopsied specimens with *H. pylori*-CagA negative; 6 = biopsied specimen without *H. pylori*; 7 = negative control

Table 2 *H. pylori* infection and the presence of CagA marker in different endoscopic findings

Endoscopic findings	Number of patients	
	H. pylori infection	CagA-positive *H. pylori* infection
Normal (n=23)[a]	3	0
Erosion (n=40)[b]	11	6
Ulcer (n=45)[b]	17	7
Others (n=7)[c]	2	2
Total (n=115)	33	15

[a] Normal gastric mucosa under endoscopy
[b] Either gastric or duodenal erosion/ulcer
[c] Patients with gallstone, hemorrhagic gastritis, annual check up

Investigation of the CagA virulence marker by the CagA specific PCR in the 33 *H. pylori*-positive samples (Table 2) revealed that 15 of them (45.5%) gave the amplified DNAs with expected size (400 bp product of the *cagA* gene) as seen in the CagA-positive isolate (Fig. 1B). In addition, the 294 bp DNA of the *glmM* gene was also observed in all the 33 *H. pylori*-positive samples (as internal controls). It was also found that the prevalence of the *H. pylori* carrying this virulence marker in the patients with peptic ulcer and erosion was not statistically different ($p > 0.05$) from that in the individuals with normal gastric mucosa.

4. Discussion

Perez–Perez *et. al.* [15, 16] reported that the seroprevalence of *H. pylori* infection in Thailand was high. However, as it was known, the serological method also detects the *H. pylori* antibody in persons whose bacterial infection has already been eradicated. So, it can not differentiate between the current and the past *H. pylori* infection with the sero-positive results.

Using an *in vitro* DNA amplification technique, Pongchairerks *et al.* reported that the prevalence of *H. pylori* infection in Thai people with gastritis and gastroduodenal ulcers was 53.8% (21 in 39) [9]. Surprisingly, our PCR result revealed that the infection of *H. pylori* in Thai patients with gastric erosion and ulcer was only 33% (28 in 85) (Table 2). Besides the non-homogeneous distribution of the bacteria in the stomach, it was speculated that the gastric lesions in the patients in this study might result from other factors such as non steroidal anti-inflammatory drugs (NSAIDs) [17].

High prevalence of the CagA positive *H. pylori* infection, determined by PCR, has been reported in many ethnic groups including Thai people. Here, we found that the prevalence of the CagA-positive strain was not as high as previously reported in Thai patients [9]. The low prevalence of the CagA-positive strain in this study possibly result from a limited number of patients. Moreover, the low prevalence was probably due to an unequal amplification of the CagA marker in each strain of the bacteria as noticed by Queiroz *et. al.* [18]. We also observed the non-association between the CagA-positive bacteria infection and the gastric pathology, i.e. the occurrence of the CagA-positive strain in the patients with gastric lesions was not statistically different from those with normal gastric mucosa ($p > 0.05$). This was concordant to the previous studies in Thai people [9] and in Singaporean individuals [19]. In contrast, gastropathological lesions has been associated with *H. pylori* CagA-positive strain in western populations [13]. Thus, it may be that

the CagA proteins are not universal virulence markers [19] and that the different gastric lesions are the results of other factors, e.g. host genetic diversity, NSAIDs. To make a definite conclusion of the association between the CagA virulence factor and the gastric lesion, more patients in each group are needed. In addition, it was previously recommended to use more than one set of primers specifically to the *CagA* gene since the gene seems to be polymorphic and might cause unexpected irregular amplification in some bacterial strains [18]. As the number of the bacteria in the stomach is varied, analysis of the virulence marker from the bacterial isolate (from culture) would give more accurate results than that from the gastric biopsied specimens. Moreover, studies of other bacterial virulence marker(s) as well as host defensive gene(s) might reveal more informative data than the analysis of only the *CagA* gene.

5. Acknowledgements

Special appreciation is given to all patients whose gastric biopsies were used in this study and to the surgical team of Thammasat Hospital for their collection of the specimens. The authors also thank Miss Sawanee Tengrungsun for her assistance in statistical analysis. The work was supported by a grant from Thammasat University.

6. References

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