Prevalence of *Helicobacter pylori* cagA genotype among dyspeptic patients in Southern Thailand

Sueptrakool Wisessombat¹*, Chatruthai Meethai²

¹School of Allied Health Sciences and Public Health, Walailak University, Tha Sala, Nakhon Si Thammarat Province, Thailand

²Faculty of Medical Technology, Prince of Songkla University, Hat Yai, Songkhla Province, Thailand

**ABSTRACT**

**Objective:** To investigate the prevalence of *Helicobacter pylori* (*H. pylori*) infection in dyspepsia patients and its relation to virulence factor cagA gene.

**Methods:** In total, 110 gastric biopsies from dyspeptic patients were comparatively studied using rapid urease test and multiplex polymerase chain reaction (PCR).

**Results:** Multiplex PCR detected three genes of 16S rRNA, cagA, and ureC. *H. pylori* was detected in 14 gastric biopsies (13%). Significantly higher numbers of females were infected. Furthermore, cagA gene was found in all *H. pylori*-positive specimens. In addition, the result indicated that the multiplex PCR with annealing temperature at 57°C was able to effectively amplify specific products.

**Conclusions:** The results confirmed high prevalence of cagA gene in *H. pylori* among dyspeptic patients in Southern Thailand.

**KEYWORDS**

*Helicobacter pylori*, Multiplex polymerase chain reaction, Dyspepsia, cagA gene

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**1. Introduction**

*Helicobacter pylori* (*H. pylori*), a Gram-negative microaerobic bacterium, is associated with human gastritis, gastric ulcer and gastric cancer[1]. Cytotoxin associated gene cagA is one of the most studied virulence factors of *H. pylori*. cagA has been proposed as a marker for a genomic pathogenicity island[2]. *H. pylori* cagA-positive strains have been observed to be more virulent than the *H. pylori* cagA-negative strains. The cagA-positive strain increases the risk of development of atrophic gastritis, mucosal inflammation, and adenocarcinoma[3].

Histology has been considered to be the gold standard for detection of *H. pylori*. However, the detection of *H. pylori* relies upon a number of gastric biopsies, staining methods, and the level of experience of the examining
pathologist\textsuperscript{[4]}. Molecular methods based on polymerase chain reaction (PCR) amplification are rapid, specific and sensitive. A number of PCR-based methods have been reported for the detection of Helicobacter\textsuperscript{[5-7]}. In Southern Thailand, the epidemiological studies on prevalence of 

\textit{H. pylori} infection are very few.

The objective of the present study was to investigate the prevalence of \textit{H. pylori} infection among dyspeptic patients in Southern Thailand. We also established a multiplex PCR for the identification of \textit{H. pylori}. In addition, \textit{cagA} gene-based multiplex PCR can simultaneously detect the presence of \textit{cagA} gene which is responsible for pathogenesis of \textit{H. pylori} infection.

\section{Materials and methods}

\subsection{Bacterial strains and culture conditions}

Reference strains including \textit{H. pylori} NCTC 11637 and \textit{H. pylori} NCTC 11638 were used for development of a multiplex PCR. Helicobacter species were cultured on Brucella blood agar (BBL, USA) with 10\% defibrinated horse blood (Oxoid, UK). Plates were incubated at 37 °C for 48 h under microaerobic atmosphere using gas pack system (Oxoid).

\subsection{Gastric biopsies}

Gastric biopsies were collected from Institute of Gastroenterology and Hepatology, Songklanagarind Hospital, Prince of Songkla University, Thailand. A total of 110 dyspeptic patients undergoing upper endoscopy were biopsied and tested for \textit{H. pylori} infection by a Campylobacter–like organism (CLO) test (Kimberly–Clark, USA) and multiplex PCR. The CLO test was performed according to the manufacturer’s instructions, and the results were interpreted after 24 h.

\subsection{Multiplex PCR}

Bacterial DNA was extracted and purified directly from biopsy specimens by QIAamp DNA Mini Kit (QIAamp, USA). The identification of \textit{H. pylori} confirmed specific primers. In this study, a multiplex PCR was designed to detect three genes of 16S rRNA, \textit{cagA} encoding for virulence factor cytotoxin associated gene A, and \textit{ureC} for housekeeping urease gene C (Table 1). PCR was performed in a total reaction volume of 25 mL containing 1× TopTaq Master (QIAamp), 1.5 mmol/L MgCl$_2$, 200 mmol/L deoxynucleotide triphosphates, 1.25 IU Taq polymerase, 20 μmol 16S rRNA primers for \textit{H. pylori}, 15 μmol each of \textit{cagA} primers and \textit{ureC} primers for \textit{H. pylori}. Amplification consisted of initial denaturation at 94 °C for 4 min, followed by amplification at 94 °C for 30 seconds, primers annealing at 50–60 °C for 30 seconds, and extension at 72 °C for 30 seconds. The samples were amplified for 40 cycles, with a final extension step at 72 °C for 5 min. PCR cycles were carried out in PTC–100, Peltier Thermal Cycler (Pegasus Scientific, USA). About 2 μL amplified products were analysed by 2% agarose (Gibco–BRL Life Technologies, USA) gel electrophoresis in Tris–Acetate–EDTA buffer at 100 V for 35 min. PCR products were visualized after ethidium bromide staining.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|}
\hline
\textbf{Target} & \textbf{Primer sequences} & \textbf{Annealing temperature} & \textbf{Amplicon size (base pair)} & \textbf{References} \\
\hline
\textbf{16S} & F 5’ TAA GAG ATU AGG CTA TAT GTC C 3’ & 56 & 534 & [22] \\
\textbf{rRNA} & R 5’ TCC CAG CAC TTA AGG GCA AT 3’ & 59 & 400 & [23] \\
\textbf{cagA} & F 5’ AAT ACA CCA AGG CCG CCA AG 3’ & 57 & 294 & [24] \\
\textbf{ureC} & R 5’ TTG TCG CCGATTGCTGCAGCA A 3’ & 39 & 26 & [22] \\
\hline
\end{tabular}
\caption{Primer sequences used in this study.}
\end{table}

\subsection{Statistical analysis}

Data were subjected to analysis of invariance. Determination of the prevalence of \textit{H. pylori} infection in relation to gender and age were carried out by Fisher’s exact test (2-tailed test). Statistical analysis was performed using the Statistical Package for Social Sciences package version 12.0 (SPSS, USA).

\section{Results}

In total, in 110 dyspeptic patients 56 were female and 54 were male. \textit{H. pylori} infected patients were evaluated for the relation of gender and age as shown in Table 2.

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|}
\hline
\textbf{Gender} & \textbf{Age (years old)} & \textbf{Total} & \textbf{H. pylori positive (\%)} \\
\hline
\textbf{Female} & <20 & 2 & 0 \\
& 21–40 & 3 & 0 \\
& 41–60 & 25 & 3 (12\%) \\
& >60 & 26 & 7 (27\%) \\
& Total & 56 & 10 (18\%) \\
\hline
\textbf{Male} & <20 & 2 & 0 \\
& 21–40 & 2 & 0 \\
& 41–60 & 22 & 2 (9\%) \\
& >60 & 28 & 2 (7\%) \\
& Total & 54 & 4 (7\%) \\
\hline
\end{tabular}
\caption{H. pylori infection rates in relation to gender and age.}
\end{table}
The results demonstrated that *H. pylori* infection rates were significantly higher (*P*<0.05) in female aged over 60 years.

The presence of *H. pylori* in the gastric biopsies was detected by CLO test and PCR. The results showed that *H. pylori* were positive in 14 gastric biopsies (13%). cagA gene was detected in all *H. pylori*-infected dyspeptic patients. Moreover, the optimal condition of the multiplex PCR was carried out with a single tube method by incorporating all specific primers. The combination of 16S rRNA, cagA, and ureC primers were able to be detected at 57 °C annealing temperatures (data not shown).

4. Discussion

It has been shown that *H. pylori* infection rate in dyspeptic patients was 13%. Nevertheless, the prevalence of *H. pylori* cagA genotype was 100%. Likewise, the positive rate for the cagA gene in *H. pylori* of dyspeptic patients was 94% in Northeast Thailand[8]. Whereas, it was reported that the prevalence of cagA gene was found to be 60%–70% in Western countries[9].

In Thailand, *H. pylori* infection rate was 34.1%[10]. Moreover, 48% of dyspeptic patients were infected with *H. pylori*[11–13]. Similarly, the prevalence of *H. pylori* infection changes considerably with age[14,15].

The *H. pylori* cagA genotype strains are associated with gastric carcinogenesis by increasing interleukin 8 secretion, NF-κB activation, and stimulation of cell proliferation[2,16,17]. The prevalence of gastric cancer in Thailand was reported to be lower than that in other South-East Asia countries even the prevalence of *H. pylori* infection was higher[18]. In Thailand, the prevalence of gastric cancer was 1.5%, while, it was 3.3% in Malaysia[19]. Furthermore, the Western type cagA was detected to be more frequently than the East Asian type in Thai dyspeptic patients. It was also found significantly more common in patients with a gastric ulcer but was not significant in gastric cancer[20]. Recent study have revealed that the variation of Western type cagA gene may be involved in the development of diseases[21].

In conclusion, these observations indicated that the cagA gene is an important virulence factor for *H. pylori*-infected dyspepsia patients. In addition, our multiplex PCR has allowed simultaneous amplification of *H. pylori* virulent genes direct from biopsies.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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**Comments**

**Background**

*H. pylori* is a Gram-negative bacterium causing human gastritis, gastric ulcer and gastric cancer. Cytotoxin associated gene A is one of the most studied virulence factors of *H. pylori*. *H. pylori* cagA–positive strains have been observed to be more virulent than the *H. pylori* cagA–negative strains. Diagnosis of *H. pylori* cagA genotype infections can be performed by PCR.

**Research frontiers**

This study was performed in order to determine the prevalence of *H. pylori* infection among dyspeptic patients in Southern Thailand. Furthermore, also this study established a new multiplex PCR for the identification of *H. pylori* using cagA gene–based PCR.

**Related reports**

The manuscript discussed the prevalence of *H. pylori* infections from the United States and Southeast Asia. To establish a new multiplex PCR, the gastric biopsies from dyspeptic patients were comparatively studied using CLO test and multiplex PCR.

**Innovations and breakthroughs**

This study indicated that the multiplex PCR with annealing temperature at 57 °C was able to effectively amplifiy specific PCR products. Moreover, the cagA gene–based PCR has allowed simultaneous directly detection of virulent genes from gastric biopsies.

**Applications**

It may be significant to know the distribution of cagA
gene in dyspeptic patients. The results of the present study confirmed the high prevalence of cagA gene in *H. pylori* among dyspeptic patients in Southern Thailand, especially in female aged over 60 years.

**Peer review**

This article is interesting and revealed that in Southern Thailand, the prevalence of cagA gene in *H. pylori* was found significantly high in female.

**References**


