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

Sueptrakool Wisessombat[1*], Chatruthai Meethai[2]

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

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

PEER REVIEW

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Assoc. Prof. Dr. Siroj Jitsuronk, Faculty of Medical Technology, Prince of Songkla University, Hat Yai, Songkhla, Thailand.
E-mail: siroj.jit@gmail.com

Comments

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

Objective: To investigate the prevalence of *Helicobacter pylori (H. pylori)* infection in dyspepsia patients and its relation to virulence factor *cag*A 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, *cag*A, and *ure*C. *H. pylori* was detected in 14 gastric biopsies (13%). Significantly higher numbers of female were infected. Furthermore, *cag*A 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 *cag*A gene in *H. pylori* among dyspeptic patients in Southern Thailand.

KEYWORDS

Helicobacter pylori, Multiplex polymerase chain reaction, Dyspepsia, *cag*A gene

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 *cag*A is one of the most studied virulence factors of *H. pylori*. *cag*A has been proposed as a marker for a genomic pathogenicity island[2]. *H. pylori cag*A-positive strains have been observed to be more virulent than the *H. pylori cag*A-negative strains. The *cag*A-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

*Corresponding author: Sueptrakool Wisessombat, School of Allied Health Sciences and Public Health, Walailak University, Tha Sala, Nakhon Si Thammarat Province, Thailand.
Tel: +66 75672472
Fax: +66 75672106
E-mail: sueptrakool.wi@wu.ac.th

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pathologist[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*[5-7]. In Southern Thailand, the epidemiological studies on prevalence of *H. pylori* infection are very few.

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

2. Materials and methods

2.1. Bacterial strains and culture conditions

Reference strains including *H. pylori* NCTC 11637 and *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).

2.2. 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 *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.

2.3. Multiplex PCR

Bacterial DNA was extracted and purified directly from biopsy specimens by QIAamp DNA Mini Kit (QIAamp, USA). The identification of *H. pylori* confirmed specific primers. In this study, a multiplex PCR was designed to detect three genes of 16S rRNA, *cag*A encoding for virulence factor cytotoxin associated gene A, and *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 *H. pylori*, 15 μmol each of *cag*A primers and *ureC* primers for *H. pylori*. Amplification consisted of initial denaturation at 94 °C for 4 min, followed by denaturation 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.

Table 1
Primers used in this study.

Target gene	Primers sequences	Annealing temperature (°C)	Amplicon size (base pair)	References
16S rRNA	F 5′ TAA GAG ATC AGC CTA TAT GTC C 3′ R 5′ TCC CAC GCT TTA AGC GCA AT 3′	56	534	[22]
*cag*A	F 5′ AAT ACA CCA ACG CCT CCA AG 3′ R 5′ TTG TTG CCG CTT TTG CTC TC 3′	59	400	[23]
ureC	F 5′ AAG CTT TTA GGG GTG TTA GGG GTT 3′ R 5′ AAG CTT ACT TTC TAA CAC TAA CGC 3′	57	294	[24]

2.4. Statistical analysis

Data were subjected to analysis of invariance. Determination of the prevalence of *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).

3. Results

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

Table 2
H. pylori infection rates in relation to gender and age.

Gender	Age (years old)	Total	*H. pylori* positive (%)
Female	<20	2	0
	21-40	3	0
	41-60	25	3 (12%)
	>60	26	7 (27%)
	Total	56	10 (18%)
Male	<20	2	0
	21-40	2	0
	41-60	22	2 (9%)
	>60	28	2 (7%)
	Total	54	4 (7%)

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%). *cag*A 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, *cag*A, and *ure*C primers were able to be detected at 57 °C annealing temperatures (data not shown).

4. Discussion

It has been showed that *H. pylori* infection rate in dyspeptic patients was 13%. Nevertheless, the prevalence of *H. pylori cag*A genotype was 100%. Likewise, the positive rate for the *cag*A gene in *H. pylori* of dyspeptic patients was 94% in Northeast Thailand[8]. Whereas, it was reported that the prevalence of *cag*A 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 cag*A 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 *cag*A 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 *cag*A gene may be involved in the development of diseases[21].

In conclusion, these observations indicated that the *cag*A 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 cag*A-positive strains have been observed to be more virulent than the *H. pylori cag*A-negative strains. Diagnosis of *H. pylori cag*A 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 *cag*A 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 amplify specific PCR products. Moreover, the *cag*A gene-based PCR has allowed simultaneous directly detection of virulent genes from gastric biopsies.

Applications

It may be significant to know the distribution of *cag*A

gene in dyspeptic patients. The results of the present study confirmed the high prevalence of *cag*A 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 *cag*A gene in *H. pylori* was found significantly high in female.

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