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Prevalence of *Helicobacter pylori cag*A Genotype Among Dyspeptic Patients in Southern Thailand

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To investigate the prevalence of *H. pylori* infection in dyspepsia patients and it relation to virulence factor *cag*A gene. In total, 110 gastric biopsies from dyspeptic patients were comparatively studied using rapid urease test and multiplex PCR. 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. The results conûrmed that high preva-lence of *cag*A gene in *H. pylori* among dyspeptic patients in Southern Thailand.

Key words: *Helicobacter pylori*, Multiplex PCR, Dyspepsia, and *cag*A gene.

Helicobacter pylori, a Gram–negative microaerobic bacterium is associated with human gastritis, gastric ulcer and gastric cancer[1]. Cytotoxin associated gene 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* cagA–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 pathologist[4]. Molecular methods based on polymerase chain reaction (PCR) ampliû-cation are rapidity, speciûcity and sensitivity. 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, *cagA* gene–based multiplex PCR can simultaneously detect the pres-ence of *cagA* gene that is responsible for pathogenesis of *H. pylori* infection.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Reference strains including *Helicobacter pylori* NCTC 11637 and *H. pylori* NCTC 11638 were used for development of a multiplex PCR. *Helicobacter* spp. were cultured on Brucella blood

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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).

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.

Multiplex PCR

Bacterial DNA was extracted and purified directly from biopsy specimens by QIAamp DNA Mini Kit (QIAamp, USA). The identification of *H. pylori* was 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 *ure*C for housekeeping urease gene C (Table 1). PCR was performed in a total reac-tion volume of 25 mL containing 1xTopTaq Master (QIAamp), 1.5 mM $MgCl_2$, 200 mM dNTPs, 1.25 U *Taq* polymerase, 20 µmol *16S rRNA* primers for *H. pylori*, 15 µmol each of *cag*A primers and *ure*C primers for *H. pylori*. Amplification consisted of initial denaturation at 94 °C for 4 min, followed by denaturation at 94 °C for 30 s, primers annealing at 50–60 °C for 30 s, and extension at 72 °C for 30 s. 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). Two 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	[8]
*cag*A	F 5′ AAT ACA CCA ACG CCT CCA AG 3′ R 5′ TTG TTG CCG CTT TTG CTC TC 3′	59	400	[9]
*ure*C	F 5′ AAG CTT TTA GGG GTG TTA GGG GTT 3′ R 5′ AAG CTT ACT TTC TAA CAC TAA CGC 3′	57	294	[10]

Statistical analysis

Data were subjected to analysis of invariance. Determination the prevalence of *H. pylori* infection rates 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).

RESULTS

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

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%)

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 in a single tube method by incorporating all specific primers. The combination of *16S rRNA*, *cag*A, and *ure*C primers were able to detect at 57 °C annealing temperatures (data not shown).

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[11]. Whereas, it was reported that *cag*A gene was found 60–70% in Western countries[12].

In Thailand, *H. pylori* infection rate was 34.1%[13]. Moreover, 48% of dyspeptic patients were infected with *H. pylori*[14-16]. Similarly, the prevalence of *H. pylori* infection changes considerably with age[17,18].

The *H. pylori cag*A genotype strains are associated with gastric carcinogenesis by increasing interleukin 8 secretion, NF–ºB activation, and stimulation of cell proliferation[2,19,20]. The prevalence of gastric cancer in Thailand was reported to be lower than that in other South–East Asia countries even the higher prevalence of *H. pylori* infection[21]. In Thailand, the prevalence of gastric cancer was 1.5% while, was 3.3% in Malaysia[22]. Furthermore, the Western type *cag*A was detected 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[23]. Recent study revealed that the variation of Western type *cag*A gene may be involved in the development of diseases[24].

In conclusion, this observations indicating 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. Part of this work was presented at 3rd International Conference on Gastroenterology & Urology, 2014[25]

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