INTRODUCTION

Helicobacter pylori is one of the most common chronic bacterial infection of humans and has a worldwide distribution. Epidemiological studies strongly suggested that more than 50% of the world’s population are colonized by H. pylori (Magraud et al, 1989). However, the prevalence of H. pylori infection varies from 10% to 90%, depending on age, geographic location, and socioeconomic status of the populations (Brown, 2000). In developing countries, the prevalence of H. pylori infection was found in more than 70% of the populations (Graham et al, 1991; Nurgalieva et al, 2002). Conversely, it was found in only 27.6% to 32.5% in developed countries (Everhart et al, 2000; Moayyedi et al, 2002). Although some infected populations harbor the organisms throughout their lives with no overt clinical symptoms, approximately 20% of infected populations manifest one of many different outcomes (NIH Consensus Conference, 1994), such as peptic ulcer (PU) disease, including gastric ulcer (GU) and duodenal ulcer (DU) (Kuipers et al, 1995), gastritis (Blaser, 1990), non-ulcer dyspepsia (Trespi et al, 1994), gastric cancer (Qiao et al, 2003), and mucosa-associated lymphoid tissue (MALT) lymphoma (Nakamura et al, 1998).

Thailand is also one of the developing countries with a high incidence of gastrointestinal (GI) symptoms and a high seroprevalence of H. pylori infection reported (Pérez-Pérez et al, 1990; Health Information Division, 1997). No previous studies regarding the prevalence of H. pylori infection in Chon Buri, Thailand have been reported. Therefore, in our study, we present the prevalence of H. pylori infection by culture and rapid urease test methods in patients with GI symptoms undergoing gastroscopy at Queen Sawang Wattana Memorial Hospital, Chon Buri.
Thailand. In addition, we also describe the association of H. pylori infection with demographic data, clinical diagnosis, and the previous histories of patients.

MATERIALS AND METHODS

Study population.

The study population was 112 patients who had GI symptoms and gastroscopy at Queen Sawang Wattana Memorial Hospital, Chon Buri, Thailand from October 2000 to January 2002. Gastroscopy findings showed 39 GU patients, 28 DU patients, and 34 gastritis patients. However, some of patients had two combined GI symptoms, including 8 patients exposed GU with gastritis, 2 patients exposed GU with DU, and 1 patient with exposed DU and gastritis. All of the patients were of the local population or were workers who has been living in the local community of Chon Buri for more than one year. Patients who had received antibiotics, bismuth, proton pump inhibitors (PPI) or had gastroscopy in the previous 2 months were excluded from the study. Written informed consent was obtained from all patients before the procedure.

Demographic data and medical history.

Demographic data, previous history of alcohol drinking, cigarette smoking, coffee drinking, water drinking, NSAIDs use, and domestic pets contact were obtained by direct interview.

Clinical specimens.

Four gastric-biopsy specimens were collected from each of the patients. Two gastric biopsies were taken within 2 cm of the pyloric sphincter (pyloric antrum) and two pieces were taken from the body of the stomach. One of specimens from the pyloric antrum and another from the body were placed in 0.5 ml of transport medium [brain heart infusion broth (BHI) (Oxoid, Basingstoke, England) supplemented with 10% horse serum and 20% glycerol] for storage at -70°C until culture. The two remaining gastric biopsies were used for the rapid urease test immediately after collection.

Rapid urease test

Pronto dry test kit (ENDO-SURG Medical Inc, Columbia) was used to perform the rapid urease test immediately after gastroscopy. The procedure was performed according to the manufacturer’s instructions. Briefly, two gastric-biopsy specimens from patients as previously described were placed onto the Pronto dry test immediately after collection. After that, they were incubated at room temperature. A positive result was indicated by a color change from yellow to red, within 1 hour.

Culture of H. pylori.

Before the procedure, all of the gastric biopsy specimens were evaluated after the identity of the patient was blinded by the director. Frozen gastric biopsies were thawed at 37°C and were homogenized under aseptic conditions. Trypticase soy agar (TSA) (BBL Microbiology Systems, Cockeysville, Maryland, USA) supplemented with 10% human blood, Skirrow’s supplement (Oxoid) (containing 10 mg/l of vancomycin, 2500 IU/l of polymyxin B, and 5 mg/l of trimethoprim lactate), 2 mg/l of amphotericin B (Bristol-Myers Squibb, Clinton, New York, USA), and 40 mg/l of 2,3,5-triphenyltetrazolium chloride (TTC) (Sigma, St Louis, Missouri, USA) was used as selective agar for primary isolation.

Briefly, 50 µl of gastric biopsy suspension was inoculated onto selective TSA and incubated under microaerobic conditions (gas pack® Mitsubishi®) at 37°C for up to 7 days. Suspected colonies were identified as H. pylori on the basis of characteristic spiral or curved gram-negative bacilli with golden colonies, positive results for catalase, oxidase, and urease tests, cephalothin and nalidixic acid discs susceptibility test.

Diagnosis of H. pylori infection.

Diagnosis of H. pylori infection was based on gastric biopsy-based methods including culture and rapid urease test. Patients with positive diagnostic results from any one of them were considered as H. pylori infection.

Statistical analysis.

The chi-square (χ²) test was used to compare the prevalence of H. pylori infection in the different categories of the explanatory variables. The significance level was set at 0.05.

RESULTS

The prevalence of H. pylori infection in the study population

One hundred and twelve patients (72 males
and 40 females) with a mean age of 53.0±16.8 years (age range, 16 to 87 years) participated in the study. Among 112 patients, the H. pylori positive results determined by culture and the rapid urease test were 50.9% (57 of 112) and 49.1% (55 of 112), respectively. The final result was considered H. pylori positive if either the culture or the rapid urease test was positive. From the mentioned criteria, the overall prevalence of H. pylori infection in the patients with GI symptoms was 58% (65 of 112).

The association of H. pylori infection with demographic data and clinical diagnosis of patients

The prevalence of H. pylori infection was defined according to the different demographic data of the patients, including gender, age, ABO blood group, educational status, household income, and clinical diagnosis. No statistically significant difference in gender, age, educational status, and household income was observed in terms of acquisition of H. pylori infection (data not shown). The prevalence of H. pylori infection decreased inversely to the economic status of the patient. The prevalence of H. pylori infection in patients who had a household income of less than 6,000 baht/month was 62.4% (33 of 53) and decreasing to be 57.6% (19 of 33), 56.3% (9 of 16), and 40% (4 of 10) in those who had household income range of 6,000-9,000, 10,000-15,000, and more than 15,000 baht/month, respectively (Fig 1).

ABO blood group in the study population did not have predictive value for H. pylori colonization (data not shown). Interestingly, three of four patients with the AB blood group were H. pylori positive. A larger sample size is required to evaluate this finding.

The prevalence of H. pylori infection was 78.6% (22 of 28), 56.4% (22 of 39), and 44.1% (15 of 34) in DU, GU, and gastritis patients, respectively. H. pylori prevalence in DU patients was statistically higher ($\chi^2=7.609$, p=0.023) than in other patients (Table 1).

The association of H. pylori infection with previous patient history

No statistically significant difference of H. pylori infection was noted in the patients with various histories, including alcohol drinking, cigarette smoking, coffee drinking, NSAID use, or domestic pets contact was found in our study (data not shown). The quality of water had a strong effect on the prevalence of H. pylori infection. The prevalence of infection among patients who usually consumed unboiled water was 61.6% compared with 30.8% among those who

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Table 1

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>No. of patients</th>
<th>No. of H. pylori infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GU</td>
<td>39</td>
<td>22 (56.4)</td>
</tr>
<tr>
<td>DU</td>
<td>28</td>
<td>22 (78.6)</td>
</tr>
<tr>
<td>Gastritis</td>
<td>34</td>
<td>15 (44.1)</td>
</tr>
</tbody>
</table>

$\chi^2$ tested, p<0.05, GU=gastric ulcer, DU=duodenal ulcer

Table 2

<table>
<thead>
<tr>
<th>Drinking water</th>
<th>No. of patients</th>
<th>No. of H. pylori infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiled water</td>
<td>13</td>
<td>4 (30.8)</td>
</tr>
<tr>
<td>Unboiled water</td>
<td>99</td>
<td>61 (61.6)</td>
</tr>
</tbody>
</table>

$\chi^2$ tested, p<0.05
usually consumed boiled water ($\chi^2=4.49$, $p=0.034$) (Table 2).

**DISCUSSION**

The prevalence of *H. pylori* infection in our study was higher than in previous studies from developing countries. There were only 19.4% and 20% of symptomatic patients from northeastern peninsular Malaysia and western Nigeria who were infected with *H. pylori* (Smith et al, 1999; Raj et al, 2001). However, the same trend was also observed in Jamaica; the study of Hinsada et al (2001) showed that a high prevalence (60%) of *H. pylori* infection was detected in sequential patients with GI symptoms diagnosed by rapid urease test, histology, and culture methods.

In Thailand, the seroepidemiological study of the Thai population showed that the *H. pylori* infection rate increased with age from 17.5% of children aged 5 to 9 years old to 55% during the third decade of life, while a peak in infection was 75% of those 30 to 49 years of age (Pérez-Pérez et al, 1990). Furthermore, a high prevalence of *H. pylori* infection has been previously reported in patients with GI symptoms in Siriraj Hospital, Bangkok, Thailand (Kachintorn et al, 1992). This report corresponds very closely with the prevalence of infection detected in our study population (63.6%). However, a previous study from northeastern Thailand showed that the prevalence of *H. pylori* infection in patients with GI symptoms was very high, up to 73.8% (Chinprasatsak et al, 1993), when compared with our study. Thus, these data indicate that the prevalence of *H. pylori* infection in symptomatic patients from other regions of the same country or other parts of the world are rather varied. Many variations, including studied populations, bacterial strains, geographic locations, the efficacy of diagnostic methods, environmental, and socioeconomic factors could be contributory factors, which make it difficult to interpret existing data across populations.

Our study also demonstrates findings consistent with other reports in Thailand (Kachintorn et al, 1992), in that *H. pylori* infection was highly associated with the presence of duodenal ulceration. Cytotoxin-associated protein encoded by the cytotoxin-associated gene (cagA) has been listed as an important virulence factor to stimulate the synthesis of interleukin (IL)-8 and IL-10 by gastric epithelial cells (Crabtree et al, 1999; Bodger et al, 2001). Many studies strongly show that cagA + *H. pylori* strains are closely associated with DU and gastric cancer (more than 80%) (Lage et al, 1995; Qiao et al, 2003), while a lower proportion were found in GU (65%) (Tee et al, 1995), duodenitis (50%) (Tee et al, 1995), gastritis (46% to 50%) (Tee et al, 1995; Lai et al, 2003), and non-ulcer dyspepsia (38%) (Tee et al, 1995). In addition, the corresponding data reviewed by Pérez-Pérez et al (1997) also indicated that the CagA seroprevalences in developing countries, especially Peru and Thailand (82.2% and 78.8%, respectively), were substantially higher than in Canada (41.9%), The Netherlands (39%), China (37.9%), and New Zealand (28.2%). Thus, a high correlation and high distribution of cagA + *H. pylori* strains from previous reports can be used to explain our suggestion that the infection of *H. pylori* in patients with GI symptoms is uncommonly associated with DU disease in Si Racha, Chon Buri, Thailand. However, we did not determine cagA status from our isolated *H. pylori* strains; further study is needed to confirm this suggestion.

Regarding drinking water, our study showed that the prevalence of *H. pylori* infection in patients who usually consumed unboiled water was significantly higher than in those who usually consumed boiled water. Similar data have been reported in many previous studies (Mendall et al, 1992; Nurgalieva et al, 2002). In London, the epidemiological study of IgG antibodies against *H. pylori* in patients attending a health screening clinic showed that a lack of hot water supply in childhood was a powerful risk factor associated with *H. pylori* infection for British adults (Mendall et al, 1992). A clean water index (CWI) based on a combination of three factors, namely consistency of boiling water before drinking, frequency of restoring and reusing water, and frequency of bathing and showering, was created and used as a simple measure of household hygiene in Kazakhstan. The study reported that the prevalence of *H. pylori* infection was inversely
H. pylori infection in patients with GI symptoms

correlated with CWI in the Kazakhstan population (Nurgalieva et al, 2002). H. pylori have been isolated from untreated municipal wastewater. The results indicate that H. pylori can survive in water and may be a potential source of H. pylori transmission (Lu et al, 2002). Thus, our findings can be used to support the hypothesis that water drinking may be a potential source of H. pylori transmission and to confirm that the fecal-oral route is feasible.

Poor socioeconomic conditions have been found to be highly associated with increased risk of H. pylori infection (Moayyedi et al, 2002). Interestingly, our study also shows that the prevalence of H. pylori infection decreased inversely with the income of patient. From this finding, we suggest that household income is an important factor which directly affects living conditions and personal sanitation.

In conclusion, our study demonstrates that the prevalence of H. pylori infection is relatively high and that H. pylori infection can be an associated factor in DU disease in patients undergoing gastroscopy at Queen Sawang Wattana Memorial Hospital, Chon Buri, and in eastern Thailand. Lower household income and the lack of boiled water are also associated factors which increase the risk of H. pylori infection.

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REFERENCES


