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Proportion of *Helicobacter pylori* Among Dyspeptic Patients Detected by Molecular Methods in a Teaching Hospital in Sri Lanka

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Abstract

Background: Infection with *Helicobacter pylori* is considered as a major cause of chronic gastritis, peptic ulcer disease and gastric cancer. More than half of the world's population is infected with *H. pylori*. In Sri Lanka various groups have reported a prevalence ranging from 3% to 70% over the last decade.

Objectives: The aim of this study was to determine the current proportion of *H. pylori* and risk factors for *H. pylori* infections.

Patients and Methods: The study was a cross sectional, descriptive study in which 100 dyspeptic patients who were required to undergo endoscopy examination were included. The study was carried out at a Teaching Hospital in Sri Lanka. In-house urease test and PCR amplification of the *glmM* gene of *H. pylori* was performed to diagnose *H. pylori* infection. A questionnaire was filled to collect socio-demographic data from the dyspeptic patients.

Results: Eighteen dyspeptic patients were positive for *H. pylori* by both in-house CLO (Campylobacter-like organism test) test and polymerase chain reaction (PCR). Ten of cases were male (18%) while eight were female (17%). There was no association between the demographic factors and risk of *H. pylori* infection.

Conclusions: The proportion of *H. pylori* infections was found to be 18% in the study population. There was no significant association with *H. pylori* and the studied demographic factors.

Keywords: Helicobacter, Dyspepsia, Risk Factors, PCR

1. Background

Helicobacter pylori infects almost half of the world's population (1) and is associated with chronic gastritis, peptic ulcer disease and also may contribute to gastric adenocarcinoma, mucosa associated tissue lymphoma (MALT) and primary B-cell gastric lymphoma (2). Epidemiological studies from different countries have shown a decline in the prevalence of *H. pylori* infection with a significant variability among different ethnicities (3, 4). The lowest prevalence rates were reported in developed countries (4, 5). Most of the countries in Southeast Asia still have a high *H. pylori* prevalence (6). This may be due to poor sanitary conditions and overcrowding leading to increased transmission. Antibiotic resistance among *H. pylori* is reported to be increasing worldwide, and is regarded as the main factor for reducing the efficacy of *H. pylori* eradication therapy (7).

Helicobacter pylori prevalence in Sri Lanka is an enigma ranging from 3 to 70% in different studies due to use of different identification methods with varying sensitivity (8, 9). A very high (70%) *H. pylori* rate was detected in 2002 in Sri Lanka using polymerase chain reaction (PCR), which is highly sensitive and specific (8). Current review of the literature on the prevalence of *H. pylori* globally shows a declining trend. It is therefore important to determine the current proportion of *H. pylori* infection in Sri Lanka considering that physicians often begin empirical therapy for patients with dyspepsia before endoscopy or investigations to detect *H. pylori*. Furthermore, in this study a highly sensitive and specific PCR was used as the detection method whereas the majority of the published studies have used biopsy urease test and histology giving a wide range of *H. pylori* prevalence, which makes it difficult to conclude the exact proportion of *H. pylori* in Sri Lanka.

2. Objectives

The aim of this study was to evaluate the current proportion of *H. pylori* infection among dyspeptic patients using PCR in a local setting.

3. Patients and Methods

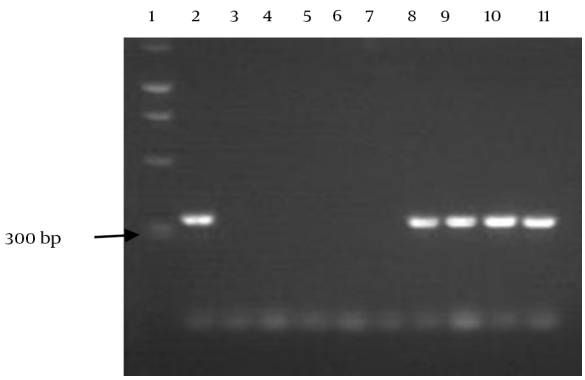
A total of 100 dyspeptic patients who had underwent upper gastrointestinal endoscopy at the tertiary reference endo-therapy unit, Colombo South teaching hospital, Sri Lanka, between August 2013 and July 2014 were included in this study. Two biopsy samples were collected from the antrum of each patient after obtaining their informed written consent. Laboratory investigations were done at the department of microbiology, faculty of medical sciences, university of Sri Jayewardenepura, Sri Lanka. Patients' demographic data was collected using an interview administered questionnaire.

One biopsy sample was immediately subjected to an in-house CLO test (10). Efficacy of the in-house biopsy test was determined by a commercially avaliable Pronto Dry® CLO test (GASTREX, France).

The second biopsy sample was kept at -80°C for PCR analysis. DNA from the biopsies were extracted using QIAamp DNA mini kit (Qiagen, Germany) following the manufacturer's instructions. The extracted DNA was amplified by PCR using primers (forward primer; AAG CTT TTA GGG GTG TTA GGG GTT T and reverse primer; AAG CTT TTA GGG GTG TTA GGG GTT T) targetting the glmM (*ureC*) gene (11).

The PCR was performed in 0.2 ml tubes using a Flexigene thermal cycler (version 31.04). The 50 µl PCR reaction mixture consisted of PCR buffer (5 µL) supplemented with 2 mM $MgCl_2$ (Go Taq Flexi DNA polymerase kit; Promega), 0.2 mM of each of dATP, dCTP, dGTP and dTTP (Promega), 0.5 µM of each primer (Integrated DNA Technologies), 1.25 U of Go Taq Flexi DNA polymerase (Promega) and 2 µL of extracted DNA (approximately 20 ng). Optimized PCR conditions were as follows: denaturation at 93°C for five minutes, PCR cycle at 93°C for one minute, 55°C for one minute and 72°C for one minute, PCR was performed for 40 cycles with a final extension at 72°C for five minutes. Polymerase Chain Reaction products were visualized on 1.5% agarose gel and resulted in a 294 base pair product (Figure 1).

Figure 1. Gel Electrophoresis of Polymerase Chain Reaction Products From *GlmM* Gene Resulting in a 294 bp Fragment



Lane 1: 100 bp DNA ladder, Lane 2: positive control, Lane 3: negative control, Lane 4 -7: PCR negative patient samples and Lane 8 - 11: PCR positive patient samples for *glmM* gene of *H. pylori*.

4. Results

Of the 100 dyspeptic patients in the study, 69 were diagnosed with antral gastritis, 15 with gastric ulcer, 10 gastric erosion and six with duodenitis, according to the endoscopic findings.

Fifty-three out of 100 cases were male and 47 were female with an age range between 20 and 86 and mean age of 50.3 years. A Total of 18 dyspeptic patients were positive for *H. pylori* by both CLO test and PCR. Of this group, ten were males (55%) while eight were female (44%). The frequency of PCR positivity for *H. pylori* was 17% (12/69) in gastritis patients, 20% (2/10) in gastric erosion patients, 13% (2/15) in gastric ulcer patients and 33% (2/6) in duodenitis patients. *Helicobacter pylori* infection was more common among male (55%) (10/18), of which the majority were in the 40 to 55 years age group. The infection rate was higher in non-Sinhalese ethnic groups (Tamils and other ethnic groups) (33%) (4/12) compared to the Sinhalese ethnic group (19%) (14/74). However, no significant association was observed between presence of *H. pylori* with patient age, gender, crowding index, education level, type of consumed water and exposure to animals (Table 1).

Table 1. Demographic Features of Dyspeptic Patients

Variable	Presence of *H. pylori*		P Value
	Positive	Negative	
Age group, y			0.61
18 - 39	6	20	
40 - 55	7	30	
> 55	5	32	
Gender			0.81
Male	10	43	
Female	8	39	
Ethnicity			0.14
Sinhalese	14	74	
Tamil and others	04	08	
Crowding index			0.29
Low	03	07	
Moderate	12	68	
Crowding	03	07	
Higher education			0.37
Yes	4	55	
No	14	27	
Water supply			0.24
Tap water	09	53	
Well water	09	29	
Exposure to animals			0.09
Yes	02	19	
No	16	63	

5. Discussion

The proportion of *H. pylori* infections was found to be low (18%) compared to the previous findings in 2002 (70%), using PCR (8). This finding is in line with the current global trend of decreasing prevalence of *H. pylori* found by previous studies (3, 12, 13). No significant association was found between *H. pylori* infection and gender, but higher frequency of infection was observed among men in the current study. Similar observations have been reported previously in China (14). The highest frequency of *H. pylori* was observed in the fourth decade of life (39%) and lowest frequency (28%) was found in patients who had already completed their fifth decade of life. In a study done in Pakistan, the frequency of *H. pylori* was highest in patients in their second and third decades of life (15). Although household crowding and increasing household contact are considered as risk factors of *H. pylori* infection, in the present study, we were unable to find any association. Furthermore, *H. pylori* infection was more frequently seen in families with three to five members (moderate family).

Although we could not find any significant association, *H. pylori* infection was less frequently observed in patients with higher education. This might be associated with good sanitary practices in this patient population. Same findings have been reported by previous studies and they have further suggested that education level may be an independent risk factor of *H. pylori* infection (1). The infection rate was higher in Tamils and other ethnic groups (33%) (4/12) compared to Sinhalese (19%) (14/74). The reason for this may be due to different culinary practices such as high oil content of food and consumption of certain spices. In 2001, Goh and Parasakthi (4) found high ethnic variability in Malaysia with our findings being in agreement with their findings. Further studies will be useful to examine the role of ethnic groups or genetic background in susceptibility to *H. pylori* infection in Sri Lanka. No significant association between *H. pylori* infection and supply of drinking water was identified in the current study.

Since *H. pylori* is capable of producing complicated gastric diseases, knowing proportion of this carcinogenic bacterium is important for the treatment of dyspeptic patients. However, the current study fails to show any increased risk of infection of *H. pylori* with the studied demographic factors among Sri Lankan patients.

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Footnotes

Authors' Contribution: Data acquisition, analysis, interpretation and drafting of the manuscript was carried out by D.L. Nushka L. Ubhayawardana; Manjula M. Weerasekera; Deepaka D. Weerasekera; T.D. Chinthika P. Gunasekera; S.S. Neluka Fernando were responsible for the study concept and design, critical revision of the manuscript and study supervision.

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