Analysis of *Helicobacter pylori* Prevalence in Chittagong, Bangladesh, Based on PCR and CLO Test

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ABSTRACT: The pathogenic bacterium *Helicobacter pylori* is a causative agent of gastric diseases in Bangladesh as well as throughout the world. This study aimed at analyzing the prevalence of *H. pylori* infection among dyspeptic patients in Chittagong, the second most populous city of Bangladesh, using 16S rRNA-based *H. pylori*-specific Polymerase Chain Reaction and *Campylobacter*-like organism test. We found that 67% of the population under study was positive for *H. pylori* infection. Gastric ulcer and duodenal ulcer disease showed statistically significant association with *H. pylori* infection; however, no association of *H. pylori* infection was observed in terms of age and gender. This study would play a crucial role in managing *H. pylori*-induced gastric diseases by understanding the current trend of *H. pylori* infection in the Chittagong region of Bangladesh.

KEYWORDS: *Helicobacter pylori*, gastric diseases, *H. pylori* 16S rRNA, PCR, CLO test

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Introduction

Helicobacter pylori is one of the most common pathogenic bacteria that infects gastric surfaces of approximately half the global human population.[1] In developing countries, 70%–90% of the population is *H. pylori* positive; almost all of these get infected early in life. In contrast, the rate of *H. pylori* infection in the developed countries is significantly lower, ranging from 25% to 50%, as a result of the improved socioeconomic standards of living over the past few decades.[2] Epidemiological reports have showed that *H. pylori* infection is very common in Bangladesh as in other developing countries.[3,4] Infection with this gastric pathogen is associated with a number of gastroduodenal diseases including acute and chronic active gastritis, peptic ulcer diseases, mucosa-associated lymphoid tissue lymphoma, and gastric malignancy.[5] Although *H. pylori* infection contributes to the development of gastric diseases, other factors, such as bacterial virulence elements, host factors, and environmental factors, play a role in the establishment of these diseases.[6] This is obvious from the fact that only about 10% of *H. pylori*-positive individuals develop associated diseases and the rate of *H. pylori* infection in patients with gastric diseases varies remarkably from one geographic location to another.[7]

H. pylori can be diagnosed in gastric biopsies by histological examination, culture, PCR, and by detection of urease activity. PCR with *H. pylori* 16S rRNA-specific primers is considerably more sensitive, faster, and less laborious than that of culture and histological examination, while the *Campylobacter*-like organism (CLO) test is also a relatively simple and efficient method for presumptive identification of *H. pylori*. CLO test is a urease-based method where *H. pylori* urease enzyme converts urea to ammonia, increasing the pH and changing the color of the indicator to phenol red.[8]

This study represents the first one in Bangladesh to detect *H. pylori* in gastric biopsy using *H. pylori* specific 16S rRNA-based PCR and CLO test together. Besides, it also analyzes the correlation of this bacterium with the gastric diseases usually linked to its presence. Moreover, age and gender aspects were evaluated and reported to *H. pylori* infection consequently. This study was carried out complying with the principles of the Declaration of Helsinki as it involved human gastric biopsy specimens.

Patients and Methods

Collection of patient samples. Gastric biopsies from 111 patients referred to gastroscopy at a hospital in Chittagong from July 2015 to November 2015 were collected. Each subject was informed about the study and written informed consent was obtained under a protocol endorsed by the National Research Ethics Committee of the Bangladesh Medical Research Council. Patients with a history of gastric surgery, active gastrointestinal bleeding or who had consumed antibiotics, proton pump inhibitors, or bismuth compounds in the previous month were excluded. Two antral

gastric biopsy specimens from each patient were taken by a specialized physician using biopsy forceps, which were cleaned with detergent and disinfected after each use. On the basis of endoscopic observations, patients were categorized as having gastritis, gastric ulcer, duodenal ulcer, or suspicion of gastric cancer, which was confirmed with extra biopsies by pathologists. Immediately after collection, the biopsy specimens were taken to the laboratory in a falcon tube with 5 mL 0.9% sterile saline solution.

CLO test. One gastric biopsy sample was introduced with a sterile pipette tip into a semisolid 2% urea agar and incubated at room temperature. Results were recorded up to 4 hours after inoculation.[9]

Genomic DNA extraction and PCR amplification of *H. pylori* 16s rRNA. Total genomic DNA was extracted from the gastric biopsies by the phenol/chloroform DNA extraction method.[10] Molecular detection of *H. pylori* was then performed on extracted DNA from biopsies by PCR using primers to amplify a 109 bp product for the *H. pylori* 16S rRNA region. Primers sequences used for the PCR include as follows: Hp1 (5′-CTG GAG AGA CTA AGC CCT CC-3′) and Hp2 (5′-ATT ACT GAC GCT GAT TGT GC-3′).[10] PCR was carried out under the following conditions: 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute and an extension time of 72°C for 5 minutes. The amplified PCR products were then electrophoresed on 1.5% agarose gel with 100 bp DNA ladder (GeneDirex, DM001-R500) and stained with ethidium bromide (500 ng/mL) to visualize the bands under a gel documentation system (WGD-30, WiseDoc). *H. pylori* DNA from a patient who was certain of being *H. pylori* positive was used as positive control. Nuclease free water (Invitrogen, 10977-015) was used as negative control.

Statistical analysis. The data were analyzed by SPSS version 16 and *P*-value was calculated using the Pearson's χ^2 test to find the significant relationship. A *P*-value of less than 0.05 was considered statistically significant.

Results

Study population. Out of 111 subjects enrolled in this study, 55 (49.5%) were males, and 56 (50.5%) were females with a mean age 42.5 ± 13.9 years (range 15–73 years old). Twenty patients (18%) had gastric ulcers, 4 (3.6%) had duodenal ulcers, 74 (66.7%) had gastritis, 5 (4.5%) had gastric cancer, and 8 patients (7.2%) were with normal endoscopic finding.

Presence of *H. pylori* infection. Patients were considered *H. pylori* positive when the gastric biopsy specimens gave positive results in any one of the two objective tests: CLO test or *Hp16s* PCR (16S rRNA-based *H. pylori*-specific PCR). Seventy-four (66.67%) of 111 patients were *H. pylori* positive. Among these, 60 (54.05%) were positive by the CLO test, 54 (48.65%) positive by PCR, and 40 (36.04%) were positive by both PCR and CLO test. Representative PCR results are shown in Figure 1.

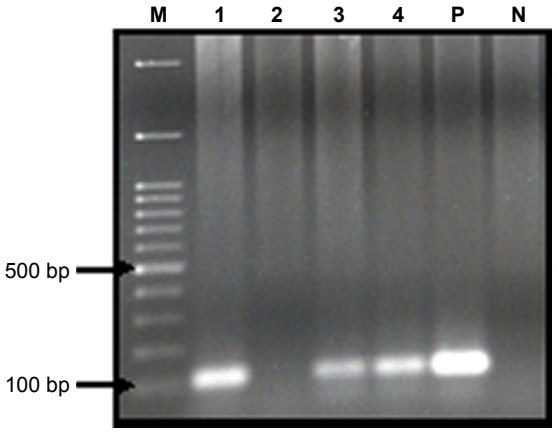

Figure 1. PCR detection of *H. pylori* using 16S rRNA primer. PCR products were electrophoresed on a 1.5% agarose gel. Lane M is a 100 bp ladder, lanes 1, 3, and 4 showed PCR products of 109 bp indicating the presence of *H. pylori*, lane 2 represented *H. pylori* negative samples. P is positive control and N is negative control.

Association of *H. pylori* infection with age and gender. In our study, patients under the age of 30 years were more likely to be *H. pylori* positive (78.3%) than the two other age strata. On the other hand, patients above the age of 40 years were more likely to be *H. pylori* negative (36.2%) compared to the two other age groups. Regarding age, 71.4% (40) of females were *H. pylori* positive compared to 61.8% (34) of males. However, there was no statistically significant association of *H. pylori* infection in relation to both age and gender ($P > 0.05$) (Table 1).

Correlation between *H. pylori* positivity and clinical outcomes. All the 74 cases being *H. pylori* positive for any of the two tests were considered for assessing the association between *H. pylori* infection and clinical presentations. It was observed that all our cases of duodenal ulcer had evidence of *H. pylori* infection, while patients with gastric ulcer had *H. pylori* in 75% of cases and the correlation between them was also proven to be statistically significant ($P < 0.05$). Interestingly, dyspeptic patients with normal endoscopic findings had *H. pylori* in 87.5% of cases and had a significant association ($P < 0.05$) with *H. pylori* positive as well (Table 2).

Table 1. Association of *H. pylori* infection with age and gender.

CHARACTERISTICS	N	*H. pylori* +VE n (%)	*H. pylori* –VE n (%)	*P*-VALUE
Age groups				
<30 years	23	18 (78.3)	5 (21.7)	0.415
30–40 years	30	19 (63.3)	11 (36.7)	
>40 years	58	37 (63.8)	21 (36.2)	
Gender				
Male	55	34 (61.8)	21 (38.2)	0.283
Female	56	40 (71.4)	16 (28.6)	

Table 2. Association between clinical outcomes and *H. pylori* infection.

ENDOSCOPIC FINDINGS	*H. pylori* +VE n = 74 (%)	*H. pylori* –VE n = 37 (%)	*P*-VALUE
Gastritis	44 (59.5)	30 (40.5)	0.104
Gastric ulcer	15 (75.0)	5 (25.0)	0.025*
Duodenal ulcer	4 (100)	0 (0)	0.046*
Gastric cancer	3 (60)	2 (40)	0.655
Normal OGD	7 (87.5)	1 (12.5)	0.034*

Note: *Statistically significant ($P < 0.05$).

However, gastritis and gastric cancer were not associated with the presence of *H. pylori* infection.

Discussion

Bangladesh is a South Asian developing country, where the rate of *H. pylori* infection is particularly high. In their serological study, Ahmad et al reported that the prevalence of *H. pylori* in Bangladesh was 92%.[11] Mahalanabis et al in a study of ^{13}C-urea breath test also reported that the prevalence of *H. pylori* was 63% in infants aged 1–3 months, 33% in 10–15-month-old children, 84% in 6–9 years old.[4] Moreover, the overall *H. pylori* prevalence in other Asian countries including, India (79% by ELISA), Pakistan (84% by PCR), and Japan (41% by measuring urinary levels of anti-*H. pylori* antibody) was also reported high.[12–14] In Europe (<40%) and the United States (<40%), a significantly lower prevalence rate of *H. pylori* was observed.[15,16] High *H. pylori* infection rates in developing countries compared to the developed world may be the consequence of poor socioeconomic conditions and unhygienic life styles.[12]

Until now, some studies have tried to show the prevalence of *H. pylori* infection in Bangladesh by serological methods, urea breath test, or CLO test. But there has not been yet any study to perform *H. pylori*-specific PCR directly on extracted DNA from gastric biopsies and CLO test together to determine *H. pylori* infection in our country. In this study, for the first time in Bangladesh, we performed PCR using *H. pylori*-specific 16S rRNA primers along with CLO test in endoscopic biopsies to determine the incidence of *H. pylori* infection in Chittagong, the port city of Bangladesh. We found that among 111 patients, 60 (54.05%) were positive by the CLO test and 54 (48.65%) were positive by PCR. Though PCR is more sensitive and more specific than the CLO test, this study produced unsatisfactory PCR results compared to the results of CLO test. This may have been due to the fact that some gastric biopsies may contain PCR inhibitors or potent nucleases as shown by Thoreson et al.[17] Besides, the small amount of gastric tissue obtained by a single biopsy or a low density of *H. pylori* on the gastric mucosa in some biopsies may produce misleading results with direct PCR from gastric biopsies.[18] Since we cannot ignore any positive sample detected by any of the two tested methods, we considered samples to be positive if any sample was detected by any of the two tests. Thus, 74 of 111 (66.67%) subjects were found to be *H. pylori* positive in our study. Given the adequate amount of gastric tissues with considerable number of bacterial cells, PCR is a better method of *H. pylori* infection than CLO test because the CLO test also has drawbacks such as inaccuracy in species-specific identification.[19]

The rate of *H. pylori* infection observed in this study demonstrated a lower percentage when compared to several previous studies as discussed earlier.[4,11] This is because there is a time interval of more than 18 years between our study and the former studies by Ahmad et al and Mahalanabis et al,[4,11] and thus rates of *H. pylori* infection might have changed during this long course of time. Another reason could be the variations in methods of studies. Unlike this study, previous studies were based on methods like serologic test and ^{13}C-urea breath test.[4,11] Moreover, those former studies were performed mainly on the population of Dhaka, the capital of Bangladesh, while our study was carried out on the population of Chittagong, the second most populous city of Bangladesh. Furthermore, we used only one biopsy tissue from gastric antrum for determining *H. pylori* prevalence, while the organism is unevenly distributed throughout the gastric mucosa; this could also be a contributing factor to the lower prevalence of our results.

Although previous studies showed that the *H. pylori* prevalence increased with age,[20,21] this study has shown a decrease in the prevalence of *H. pylori* in the older age groups. It was observed that *H. pylori* prevalence was higher in patients under the age of 30 years (78.3%) than in patients with ages between 30 and 40 years and over 40 years (63.3%); this could be due to a decline in the specific serological response among older patients and/or to a reduced number of microbes as a result of gastric atrophy.[22] Another reason could be the fact that young people in the city are more exposed to unhygienic environment, crowd, and are less aware of drinking pure water than people of older ages. However, we found no statistically significant association between *H. pylori* prevalence and age. Though *H. pylori*-positive cases in this study were more for women than men, it did not reach statistical significance corroborating other studies.[21,23]

In our study, though gastritis was the most common endoscopic finding among patients with *H. pylori* infection, no significant association was observed between the *H. pylori* infection and gastritis. There was also no association between *H. pylori* prevalence and gastric cancer. However, there was a significant association of *H. pylori* positivity with gastric ulcer and duodenal ulcer ($P < 0.05$), which agrees with the data in the literature.[5,24] Therefore, in accordance with the previous study, this study also recognizes *H. pylori* to be a main cause for peptic ulcer diseases in Bangladesh. Moreover, dyspeptic patients with normal endoscopic findings had *H. pylori* in 87.5% of cases and also had a significant

association ($P < 0.05$) with being *H. pylori* positive. This finding suggests that *H. pylori* infection is very common in Bangladeshi population.

The data of the present study may not be a precise estimate of the prevalence of *H. pylori* infection in Chittagong because the sample we studied was not adequately large. Hence, further large-scale studies are required to have a better understanding of the epidemiology of *H. pylori* infection and its association with disease outcomes in this region as well as other regions of Bangladesh.

We conclude that more than half of the dyspeptic patients in Chittagong are having *H. pylori* infection and the peptic ulcer diseases (gastric ulcer and duodenal ulcer) are significantly associated with *H. pylori* infection. This study would play a contributory role in effective diagnosis and treatment of patients with various *H. pylori*-induced gastric diseases by understanding the current trend of *H. pylori* infection in the Chittagong region of Bangladesh.

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Ethical Approval

This study was conducted under the ethical permissions granted by the Ethical Review Committee of Bangladesh Medical Research Council for Health, Population and Nutrition Sector Development Project (HPNSDP) of the Government of Peoples Republic of Bangladesh.

Author Contributions

Designed the study: AMH, MJA, and MA-F. Performed the experiments: AMH, MJA, and BR. Carried out endoscopies, biopsy collection, and patient evaluation: MAQ. Analyzed the results and drafted the manuscript: AMH. Made critical revisions and approved the final version: MA-F. All the authors read and approved the final manuscript.

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