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Smoking, Proton Pump Inhibitors and Antibiotic Administration as Factors Affecting Direct Screening of Helicobacter Pylori Infection Among Patients With Dyspepsia

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Background: *Helicobacter pylori* diagnostic tests can be affected by different interventional factors. We studied the effects of smoking, proton pump inhibitors (PPIs) and antibiotic administration on results of the bacterial culture, and its diagnosis tests due to polymerase chain reaction (PCR) and rapid urease test (RUT) methods.

Objectives: This study was aimed to investigate the undesirable effects of PPIs and antibiotics on the results of *H. pylori* screening tests in patients with gastritis.

Patients and Methods: A total of 100 patients with gastritis and indication for upper gastrointestinal (GI) endoscopy were enrolled in this study. Three biopsy samples from each patient were immediately processed for detection of *H. pylori* based on culture, RUT, and PCR methods. The sensitivity of these three detection methods was measured in the three infected patients groups that were subjected to conventional therapy, proton pump inhibitors (PPIs) therapy, and no medication as control group. The possible effects of PPIs and antibiotics on *H. pylori* detection were analyzed in vitro.

Results: The prevalence of *H. pylori* infection was higher among the non-users and PPI users (40% and 57.9%, respectively), while the number of isolated bacteria from the patients with a history of recent antibiotic prescription was significantly lower (18.75%) ($P < 0.05$). An inverse association was found between *H. pylori* infection and smoking. Among the studied methods, PCR showed the highest sensitivity in all groups. The results of RUT illustrated a significant difference between the PPI users and patients with a history of recent antibiotic administration that was consistent with the results of in vitro study ($P = 0.01$).

Conclusions: This study revealed a lower sensitivity of common *H. pylori* screening tests during the antibiotic or PPI administration. PCR was determined as the most accurate test used for diagnosis of *H. pylori* infections.

Keywords:Helicobacter pylori; Smoking; Proton Pump Inhibitors; Antibacterial Agents

1. Background

H. pylori infection is associated with gastritis, duodenal and gastric ulcers, mucosa-associated lymphoid tissue (MALT) and non-Hodgkin lymphomas (NHLs) and is a risk factor for gastric cancer (1-3). The global prevalence and incidence of this infection is different, which depends on the socioeconomic status and sanitation conditions (4). Smoking, dietary regimen, ethnicity, drug administration history, and health status are among the common risk factors for this infection. Utilization of reliable diagnostic methods plays a crucial role in management of patients who are suspected to infection with *H. pylori*. There are several methods used for diagnosis of *H. pylori* infections, which are classified into two broad categories: invasive methods, which require endoscopy, and the minimally or non-invasive methods (5). The invasive methods, in which the biopsy sampling is needed, include growth and culturing of bacteria , RUT or CLO test (Campylobacter-Like Organism test), PCR, fluorescence in situ hybridization (FISH), direct gram staining and histological tests, while the non-invasive methods include immunological methods, urea breath test (UBT) and *H. pylori* stool antigen assay (HpSA) (6, 7). The non-invasive methods are often considered as the first line diagnostic methods, but the

Implication for health policy/practice/research/medical education:
Smoking and medications, including proton pump inhibitors and antibiotics can affect the sensitivity of common screening tests for *Helicobacter pylori*. Smoking and medications should be discontinued before performing H. pylori diagnostic tests. The non-invasive diagnostic tests could be practical means of testing for *H. pylori* in patients with no recent history of medication and smoking.

sensitivity and specificity of these methods vary according to their entities and host conditions (8-10).

Eradication of *H. pylori* generally leads to ulcer healing in patients with *H. pylori*-positive peptic ulcers. Most of the patients with gastro-esophageal diseases use PPI drugs and antibiotics to control their clinical symptoms. However, this strategy is not suitable for gastric diseases induced by non-steroidal anti-inflammatory drugs (NSAIDs). Consequently, successful treatment of these patients needs correct diagnosis of the disease etiology. In this regard, administration of unprescribed drugs by patients during the clinical diagnostic processes could be problematic (8). *H. pylori* urease tests, including both invasive (RUT) and non-invasive (UBT) types, are among the best rapid detection methods for confirming the infection after examination. Urease activity of this bacterium depends on the H^+-gated urea channel that could be affected by PPIs (11, 12). Currently, all the standard therapeutic regimes contain a PPI. Long-term administration of PPIs by patients with *H. pylori* infection can reduce the stomach acid secretion, which decreases the density of colonized *H. pylori* strains (6, 13,14). Antibacterial activity of PPIs, besides the inhibitory effects of antibiotics could affect the results of these diagnostic tests.

2. Objectives

This study was aimed to investigate the undesirable effects of PPI and antibiotic administration on *H. pylori* screening tests in patients with dyspepsia.

3. Patients and Methods

3.1. Sample Collection and Patients

During August 2010 to June 2011, a total of 200 specimens from 100 patients (two biopsy specimens from each patient) undergoing upper gastroduodenal endoscopy with various dyspeptic symptoms were included in this study after obtaining the patients written consent forms and collecting demographic data. The patients were divided into three groups including those who underwent conventional therapy, PPI therapy, and those did not administer any medication as control group. Upper gastrointestinal endoscopy was performed on patients who were fast for at least 10 hours. Behaviors like smoking, and medications like PPIs and antibiotic administration were recorded for each case. The fresh biopsy specimens were analyzed by RUT and transported to the laboratory for further examinations as below.

3.2. Rapid Urease Test

To investigate *H. pylori* urease activity in the biopsy samples, RUT was performed according to the method described by Asadzadeh et al. (15). Accordingly, one fresh biopsy sample from each patient was inoculated into RUT medium. Periodic checks of the samples were made during their 48 hours incubation at 37ºC. Appearance of a pink-red color during the 48-hour study, compared with the negative controls, was considered as a positive result.

3.3. H. pylori Culture and Identification

The biopsies were immediately put into tubes containing semi solid thioglycollate as a transfer medium and sent to the laboratory. The biopsy specimens were subjected to bacterial culturing to detect the *H. pylori* under controlled conditions. Brucella agar base medium (Merck, Germany) supplemented with 7% horse blood, 10% fetal calf serum and amphotericin B (5 mg/L), trimethoprim (5 mg/L), vancomycin (10 mg/L) and polymyxin (0.25 mg/L) were used to culture the homogenized samples. The cultures were kept under a microaerophilic atmosphere (5% O_2, 10% Co_2, 85% N_2), provided by CO_2incubator innova CO-170, at 37 °C. The plates were inspected daily for the bacterial growth for a duration of 10 days. The isolates were initially identified as *H. pylori* by analysis of their colony morphologies and positive reactions for oxidase, catalase and urease tests.

3.4. DNA Extraction and PCR

To study the presence of *H. pylori*, total DNA was extracted from the gastric biopsy specimens using QIAamp tissue DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Molecular analysis of the grown colonies was also performed on the extracted DNAs of the bacterial isolates after DNA purification. Amplification of the *ureC* fragment (*glmM*) (296 bp) as a species specific gene marker for *H. pylori* was carried out using the primers shown in Table 1. PCR was performed in 25 μL reaction mixture containing 10X PCR buffer, 500 nM of each primer, 2 mM $MgCl_2$, 200 μM each dNTP, 1 U Taq DNA polymerase, and 200 ng DNA sample under the following conditions: initial denaturation of 95 °C for 1 minute followed by 30 cycles of 45 s at 95 °C, 60 s at 57 °C, and 1 minute at 72 °C, and a final extension of 5 minutes at 72 °C. A volume of 7 μL of each PCR mixture was subjected to agarose gel electrophoresis (1.2%) and ethidium bromide staining was done to detect the amplified DNA products.

In vitro inhibitory effects of PPIs and antibiotics on *H. pylori* urease activity and PCR anti-urealytic activities of omeprazole (20 mg/L) and antibiotics amoxicillin (0.12 mg/L) and metronidazole (8 mg/L) were studied on clinical isolate OC80. The assay was done in microtiter plates using a 0.5 McFarland standard inoculum of the strain in RUT medium according to the method described by Hirota et al. (16). Duplicate independent studies were carried out. Adverse effects of these drugs on RUT were assessed by following the absorbance changes at 405 nm after 2, 5 and 24 hours, which were measured by an ELISA reader (ELx808 BIO-TEK. Instruments, USA). Possible interventional

Table 1. Primer Sequences Used for Identification of *H. pylori* in the Biopsy Specimens

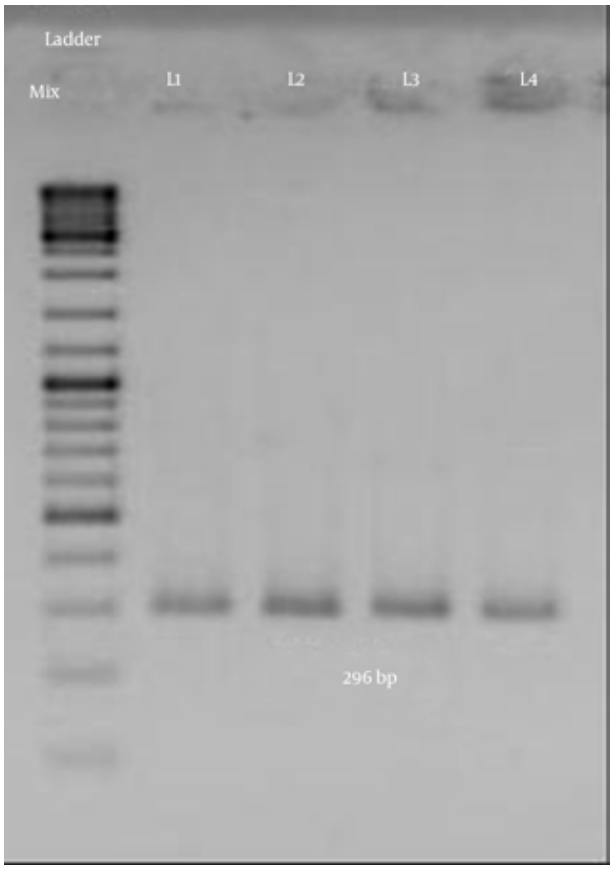
Target Gene	Primer	Primer Sequence	Amplicon Size, bp
glmM, ureC	GlmM2-F	5'-GGATAAGCTTTTAGGGGTGTTAGGGG-3'	294
	GlmM1-R	5'-GCTTACTTTCTAACACTAACGCGC-3'	

Table 2. *H. pylori* Status Based on Different Screening Tests Among the Dyspeptic Patients with Distinct Therapeutic Histories [a]

H. pylori, Screening Tests	Positive PCR, *glmM*, No. (%)	Positive RUT, No. (%)	Positive Culture, No. (%)	Decreased Sensitivity for RUT, %
Patients without any medical administration (n = 65)	27 (41.5)	26 (40)	26 (40)	1.5 and 0
PPI users (n = 19)	13 (68.4)	12 (63.5)	11 (57.9)	4.9 and 5.6
Antibiotic users (n = 16)	4 (25)	3 (18.75)	3 (18.75)	6.25 and 0

[a] Decreased sensitivity of RUT results was measured and compared with the results of the PCR and culture methods in each group.

Figure 1. Detection of *H. pylori* Isolates Based on PCR Amplification of *glmM*



Lane 2-4: PCR product of *glmM* in patients with dyspepsia

effects of these drugs on integrity of the extracted DNAs were also analyzed using PCR (*glmM*) on the treated DNA samples under the same condition.

4. Results

Out of the 100 biopsy specimens collected from patients with different gastrointestinal disorders, 40 samples had positive culture results for *H. pylori*. All of the isolates showed positive results for catalase, oxidase, urease and PCR tests (Figure 1). The samples were related to patients with different ethnicities and ages. The infected patients comprised of 15 males and 25 females of the age ranged from 10 to ≥ 70 years, with the mean age of 40-50 years. To analyze any significant effects of antibiotics or PPIs on the sensitivity of noted *H. pylori* detection tests, these samples were classified into three groups (Table 2). With respect to the examined tests, PCR showed the highest sensitivity for detection of *H. pylori* infection in all study groups. While infection rate of *H. pylori* was high among the PPI users (57.9%) and patients with no medication (40%), isolation rate of the bacterium was significantly lower among the patients with a history of recent antibiotic prescription (18.75%) ($P < 0.05$). The comparison of the RUT and PCR results showed the lowest difference in patients with no recent history of medicine administration (sensitivity of 96%) and highest ones among the patients who taking PPIs or antibiotics (sensitivities of 92% and 75%, respectively) (Table 2).

The in vitro inhibitory effect of omperazole, metronidazole and amoxicillin on urease activity of the *H. pylori* strain OC80 was only confirmed in the test samples containing omeprazole. Study of the effects of amoxicillin, metronidazole and PPI on the PCR results showed no interfering activities under the same condition.

Results of the study on both smoker and non-smoker patients showed adverse effect of smoking on colonization of *H. pylori* and its detection in the biopsy samples. Based on culture, PCR and RUT results, *H. pylori* infection among the smokers was 36.2% lower than non-smokers (Figure 2). While it was estimated that smoking can decrease the sensitivity of RUT by 5%, no statistically significant differences were found among the three detection methods in the smoker patients.

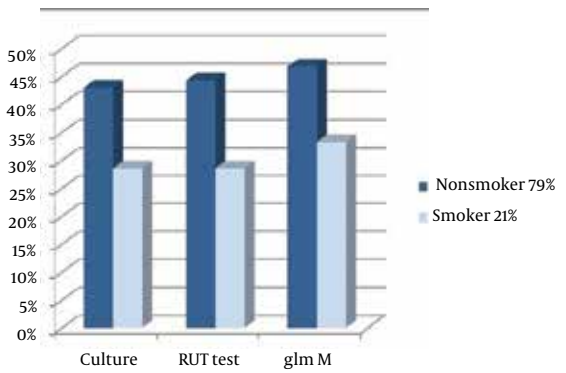


Figure 2. Comparative Results for the Three Detection Tests Used for Analysis of *H. pylori* Infection in Smoker and Non-smoker Patients

5. Discussion

H. pylori detection is not easy because of its ecological niche and fragile nature. Although there are attempts towards precise diagnosis of *H. pylori* infection by non-invasive methods, most of the common detection tests are invasive ones. The urease tests (e.g. UBT and RUT) rely on identification of *H. pylori*'s urease activity and consider as the common tests that are used in this regard. PCR is widely used for the diagnosis of *H. pylori* infections as well as analysis of its diversity, virulence properties, and resistance patterns (17). However, this method has some limitations, such as dependence on DNA quality, presence of PCR inhibitors, DNA contamination during endoscopy, and site of biopsy preparation. Furthermore, it could not differentiate live bacteria from the dead ones (18). Urease tests have been widely used because they are cheap and easy to use. Sensitivity of this test also depends on the type of urease media, concentration of urea, incubation temperature, size of biopsy, buffering capacity, bacterial load, and drug administration (18). Lack of attention to these factors could lead in false results. In the present study we examined adverse effects of smoking, and PPIs or antibiotics on detection of *H. pylori* infections in patients with different gastroduodenal disorders. False negative results was detected in RUT on the positive biopsy specimens collected from the PPI users. This finding was previously supported by Chey et al. and Graham et al. (14, 19, 20). Although there are some clinical evidences in this subject, but exact mechanism of this interfering activity has not been found yet. Nagata et al. showed that lansoprazole and omeprazole can potently and selectively inhibit the *H. pylori* urease activity and suggested that this inhibition may be related to their selective activity against the growth of this bacterium (4). This inhibitory activity was expected to result by forming disulfide bonds between the drug and active site of the enzyme (21, 22). It was also shown that UreI protein isolated from *H. pylori* functions as an H^+-gated urea channel for regulation of the urease activity, which is essential for gastric survival and colonization of *H. pylori* under acidic conditions (12). Homology of this channel with gastric H, K-ATPase that is blocks with PPIs in gastric tissue could provide further evidence to support this inhibitory effect. In our study, lack of color changes in RUT medium containing omeprazole supports this hypothesis. Statistical analysis of our results showed significant associations between sensitivity of RUT or bacterial culturing and administration of therapeutic regimens containing antibiotics or PPIs ($P < 0.05$). Antibiotic administration during endoscopy can decrease the number of metabolically active *H. pylori* strains colonizing the stomach lumen, which can affect the results of pathological, bacteriological or biochemical tests (8). According to our findings, the impact of antibiotic usage on sensitivity of *H. pylori* detection tests was dramatically higher for RUT in compare to the PCR results. The decreased sensitivity of RUT in antibiotic users was greater than PPI users ($P = 0.01$). This inhibitory effect was not confirmed by the in vitro results. The presence of metabolically inactivated bacteria in the gastric tissue during antibiotic therapy could explain this result (18).

In the current study, smoking was negatively associated with *H. pylori* infection. Inverse association of smoking and *H. pylori* infection was previously reported by two studies (23, 24). However, there are some other controversial results from other studies (25-28). Changes in gastric microenvironment through smoking, such as increased acid and mucin secretion and gastric carcinogenesis may explain these results (29). However, a study on larger population is needed to establish this association. RUT results of the smokers showed 5% decreased sensitivity compared with the PCR and bacterial culture findings. Although avoiding of smoking four hours before UBT was proposed by some companies, but no study described adverse effect of smoking on detection of *H. pylori* infection based on RUT. Reduced colonization rate or metabolic activity of *H. pylori* among the smokers could be explained by changes in gastric histology or reduced inflammation and hence decreased nutrient supplies in their gastric body (30). This reduced metabolic activity could be associated with reduced sensitivity of common screening tests for detection of *H. pylori* infection.

In conclusion, the results of this study showed reduced sensitivity of common screening tests among the patients with recent medication history and also among the smokers. These results also provide initial evidence about inverse association between *H. pylori* colonization and smoking. Our results collectively suggested discontinuing use of treatment regimens or hindering of smoking before performing common *H. pylori* screening tests.

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Authors' Contributions

Tabassom Mirzaei and Masoud Alebouyeh designed the study and wrote the manuscript. Tabassom Mirzaei performed the laboratory experiments. Leila Shokrzadeh cultured the biopsy samples. Nastaran Farzi revised the manuscript. Hamid Asadzadeh Aghdaei, Homayoun Zojaji and Mohammad Reza Zali provided the biopsy samples and examined the patients.

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