Phenol Red Chromoendoscopy for *Helicobacter pylori* Detection

Mohamed Abdel-moghny Moustafa[1], Sameh Ahmed Abdel-bary[1] and Ashraf Amin Abdel-Aziz[2]

[1]Internal Medicine Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[2]Tropical Medicine Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt
samehmind@gmail.com

Abstract: Background: Chromoendoscopy or tissue staining involves topical application of stains or pigments to improve localization, characterization, or diagnosis during endoscopy. Phenol red staining has been used to detect and map the distribution of *H. pylori*. **Objectives:** The aim of this study was to investigate the value of phenol red chromoendoscopy in *H. pylori* diagnosis compared with histopathology as gold standard. **Patients and methods**: a total of 80 adult patients with dyspepsia were enrolled in the study. Patients on proton pump inhibitors or H2 blockers treatment up to one month before the endoscopic study, those on *H. pylori* eradication therapy up to 6 months before the endoscopy and patients with gastric surgery were excluded. Patients underwent upper gastrointestinal endoscopy and phenol red chromoendoscopy. Gastric biopsies were taken either randomly from antrum and body in negative chromoendoscopy cases (yellow staining) or as directed by chromoendoscopy in positive chromoendoscopy cases (red staining). Biopsies were examined after hematoxylin and eosin staining for *H. pylori* detection. **Results:** The study included 38 male patients (47.5%) and 42 female patients (52.5%) with their ages ranged between 19-56 years and mean age of 35.8±8 years. According to histopathological examination, 71 patients (88.75%) were *H. pylori* positive and 9 patients (11.25%) were negative. 65 patients (81.25%) were positive for *H. pylori* by phenol red chromoendoscopy, while 15 patients (18.75%) were negative. Area under the receiver operating characteristic curve (AUROC) for phenol red chromoendoscopy was 0.895 (95% CI 0.806 - 0.952, P =0.0001) with concordance correlation coefficient of 0.6121 (95% CI 0.4639- 0.727).The test had 90.1% sensitivity, 88.9% Specificity, 98.5% positive predictive value (PPV), 53.3% negative predictive value (NPV), 90% accuracy, 8.11 positive likelihood ratio and negative likelihood ratio of 0.11. **Conclusion:** Phenol red chromoendoscopy is a useful method for *H. pylori* detection that had immediate reading and can be used in patients with contraindications for biopsy or to direct the biopsy taking in focal and scattered infection.
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1. Introduction:

Infection with *Helicobacter pylori* is a global health problem affecting 20–50% of the western world's population and up to 80% of the population in developing countries (1). Presence of *H. pylori* is known to be associated with a wide range of gastrointestinal disorders including peptic ulcer, gastric carcinoma, and mucosa-associated tissue lymphoma, thus the ability to correctly diagnose and eradicate the pathogen is important for managing these diseases(2).

Invasive diagnosis of *H. pylori* includes histopathological examination of endoscopic biopsies. Although achieving sensitivity and specificity of >95% in *H. pylori* diagnosis, the detection of *H. pylori* by histopathology relies upon a number of issues including the site, number, and size of gastric biopsies, method of staining, and the level of experience of the examining pathologist (3).Also as the prevalence and density of *H. pylori* varies throughout the stomach, particularly in the face of medications that may reduce the density of *H. pylori*, random biopsies may miss diagnosis (4).

Chromoendoscopy refers to the topical application of stains at the time of endoscopy in an effort to enhance tissue characterization, differentiation, or diagnosis (5).The use of chromoendoscopy in the gastrointestinal tract was first described in 1977 (6) and it has been used in the evaluation of Barrett's esophagus (7), esophageal adenocarcinoma (8), gastric metaplasia and adenocarcinoma (9), colon polyps (10), cancer colon (11), and surveillance in inflammatory bowel disease (12).

Phenol red is a pH indicator. It detects alkaline pH by a color change from yellow to red. A promising clinical application of phenol red is the detection of *Helicobacter pylori* infection. The urease produced by the bacterium catalyzes hydrolysis of urea to NH_3 and CO_2, resulting in an increase in pH. As a result, *H. pylori* can be observed in red stained mucosa after phenol red chromoendoscopy (13). This endoscopic

procedure is a relatively easy method for detecting *H. pylori* infection and is not harmful to humans (14).

Aim of the work:

The aim of this study is to investigate the value of phenol red chromoendoscopy in *H. pylori* detection compared with histopathology as a gold standard diagnostic method.

2. Patients and methods:

This study was carried out on 80 patients with dyspepsia referred for upper gastrointestinal endoscopy at the gastrointestinal endoscopy unit, Ain Shams University Hospital.

Patients on proton pump inhibitors or H_2 blockers treatment up to one month before the endoscopic study, those on *H. pylori* eradication therapy up to 6 months before the endoscopy and patients with gastric surgery were excluded.

Informed consent was obtained from each patient included in the study and the study protocol confirms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Upper gastrointestinal endoscopy was carried out for all patients using Olympus XQ-30 endoscope, Olympus Co., Tokyo, Japan with forward vision. Gastric juice was aspirated to improve visibility during endoscopy. Chromoendoscopic staining, using a spray type catheter (PW-5L-1; Olympus Co., Tokyo, Japan) was done.20 ml of phenol red at 0.1% concentration were instilled over the mucosa of the gastric cavity in a homogeneous way in all patients. A minute was waited to visualize the reaction of the mucosa to the application of the dye. Red staining of the mucosa either diffuse or focal indicates a positive reaction while yellow staining indicates a negative reaction (Figure 1).

Multiple biopsy samples of gastric mucosa were taken, using standard biopsy forceps from zones with positive staining (red). For patients with negative staining (yellow), 4 quadrant biopsy samples of both the antrum and the body were taken. The samples were examined histopathologically using hematoxylin and eosin stain.

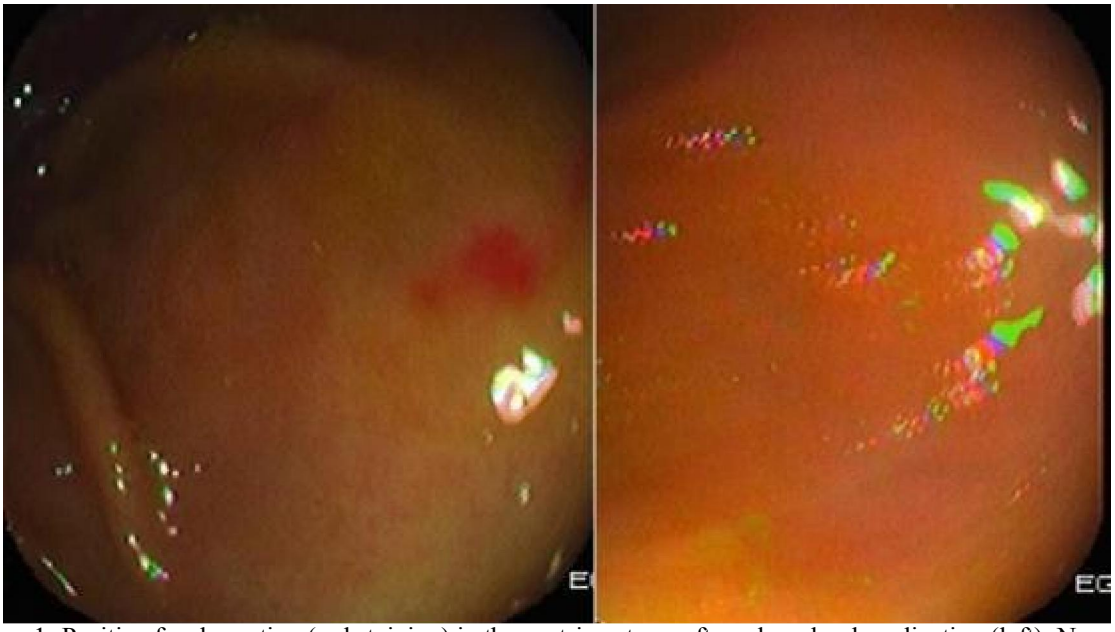

Figure 1- Positive focal reaction (red staining) in the gastric antrum after phenol red application (left). Negative reaction (yellow staining) after phenol red application in the antrum (right).

Statistical Analysis:

Data was collected and statistically analyzed using SPSS v.18.0 (IBM Corp., Armonk, NY, USA). Quantitative variables were described as mean ± SD while qualitative variables were described as frequency and percentage. With histopathology as gold standard, the receiver operating characteristic curve (ROC) for phenol red chromoendoscopy was constructed and area under the receiver operating characteristic curve (AUROC) calculated. Also sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, positive likelihood ratio, negative likelihood ratio and diagnostic odds ratio were calculated. Concordance correlation coefficient (kappa coefficient) between the two tests (histopathology and phenol red chromoendoscopy) was estimated. P value < 0.05 was used to indicate statistical significance.

3. Results:

A total of 80 patients were included in the study. Thirty eight patients were males (47.5%) and 42 patients were females (52.5%). Patients' ages ranged between 19-56 years with a mean age of 35.8±8 years (Table 1).

The most common endoscopic findings in the studied patients were gastritis in 56 patients (70%) and normal endoscopic findings in 22 patients (27.5%). Duodenal ulcer was found in one patient (1.25 %), hiatus hernia (HH) in 2 patients (2.5%), gastric ulcer (GU) in 2 patients (2.5%) and GERD in 4 patients (5%) (Table1).

According to histopathological examination, 71 patients (88.75%) were *H. pylori* positive and 9 patients (11.25%) were negative. Phenol red chromoendoscopy examination revealed that 65 patients (81.25%) were positive for *H. pylori*, while 15 patients (18.75%) were negative (Table 1).

Table (1) the study population characteristics

Parameter	Findings	
Age	35.8±8	19-56 years
Sex	Male Female	38 (47.5%) 42 (52.5%)
Endoscopic findings	Normal Gastritis Duodenal ulcer Gastric ulcer Hiatus hernia GERD	22 (27.5%) 56 (70%) 1 (1.25%) 2 (2.5%) 2 (2.5%) 4 (5%)
Histopathological examination	Positive Negative	71 (88.75%) 9 (11.25%)
Phenol red chromoendoscopy	Positive Negative	65 (81.25%) 15 (18.75%)

With histopathological examination as gold standard, phenol red chromoendoscopy test had one false positive result, 7 false negative results, 8 true negative results and 64 true positive results (Table 2).

Table (2) Phenol red chromoendoscopy compared with histopathological examination

Phenol red chromoendoscopy	Histopathological examination	
	Positive	Negative
Positive	64	1
Negative	7	8

Area under ROC curve (AUROC) for phenol red chromoendoscopy was 0.895 (95% CI 0.806 - 0.952, *P* =0.0001) (figure 2). Phenol red chromoendoscopy had 90.1% sensitivity, 88.9% Specificity, 98.5% positive predictive value (PPV), 53.3% negative predictive value (NPV) and 90% accuracy. Its positive likelihood ratio (PLR) is 8.11 and its negative likelihood ratio (NLR) is 0.11.Diagnostic odds ratio for the test was 1.8205 (95 % CI 0.75- 4.44).The concordance correlation coefficient for the test compared with histopathology was 0.6121 (95% CI 0.4639-0.727) (Table 3).

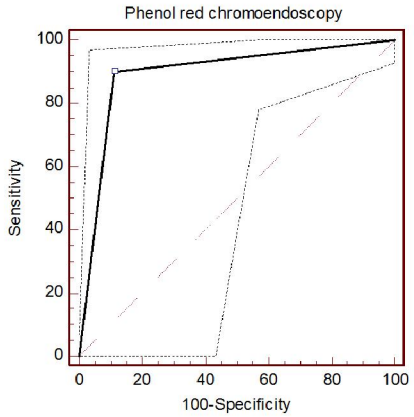

Figure 2- Phenol red chromoendoscopy ROC curve

Table (3) Performance of phenol red chromoendoscopy test for *H. pylori* detection

Variable	Findings	
AUROC	0.895	95% CI 0.806 - 0.952 (*P* =0.0001)
Sensitivity	90.1%	95% CI 80.7 - 95.9
Specificity	88.9%	95% CI 51.7 - 98.2
PPV	98.5%	
NPV	53.3%	
PLR	8.11	
NLR	0.11	
Accuracy	90%	
Odds ratio	1.8205	95 % CI 0.75- 4.44
Concordance correlation coefficient	0.6121	95% CI 0.4639-0.727

4. Discussion:

Noninvasive tests for *H. pylori* diagnosis, such as urea breath test (UBT), are as accurate in diagnosing *H. pylori* status as invasive tests in untreated patients (15).However, as the incidence of gastric cancer is closely associated with *H. pylori* infection, it is important in some cases to use invasive endoscopic examination with biopsies and histopathological evaluation of *H. pylori* status (16, 17). As *H. pylori*

infection is usually unevenly distributed across the gastric mucosa, sampling errors leading to false negative results are unavoidable (18).

The use of phenol red for the diagnosis of *H. pylori* infection was initially described by Kohli *et al.* who used it to assess infection distribution in the gastric mucosa (19). After that, Kato *et al.* (20) assessed the distribution of *H. pylori* in the gastric mucosa of 185 patients with histologically established gastric cancer, using both the Campylobacter-like organism (CLO) test and phenol red dye spraying.

The current study aimed to investigate the value of phenol red chromoendoscopy in *H. pylori* detection compared with histopathology as a gold standard diagnostic method. Area under the receiver operating characteristic curve (AUROC) for phenol red chromoendoscopy was 0.895 (95% CI 0.806 - 0.952, *P* =0.0001) with concordance correlation coefficient (kappa coefficient) of 0.6121 (95% CI 0.4639- 0.727). The test had 90.1% sensitivity and 88.9% Specificity.

Using histopathology as gold standard, Kohli *et al.* reported the sensitivity and specificity of phenol red dye spraying for *H. pylori* diagnosis to be 100% and 85% respectively in their 108 patients (19).

Iseki *et al.* (13) showed that dye's sensitivity and specificity to detect h pylori were 95% and 92%, respectively in sixty five patients with early gastric cancer before their operations.

On the other hand, Mitsuhashi *et al.* (21) reported a sensitivity and specificity of 74.3% and 100%, respectively in a sample of 82 surgically resected stomachs with early gastric carcinomas.

Cho *et al.* in Korea reported phenol red chromoendoscopy sensitivity of 81.3% and specificity of 81.5% with histopathological examination as gold standard. Comparing the results with urea breath test (UBT) as gold standard , phenol red staining showed sensitivity of 84.4% and specificity of 74.1%.(22).

With histopathology as gold standard, the study by Ahumada *et al.* (23) on 160 patients revealed that phenol red chromoendoscopy had a sensitivity of 91% and specificity of 89% with Kappa coefficient of 0.73. While Hernández-Garcés *et al.* (24) study in Cuba on 195 patients showed a phenol red chromoendoscopy sensitivity of 72.6% and specificity of 75.5% with Kappa coefficient of 0.4.

Our study also revealed a positive predictive value (PPV) of 98.5%, negative predictive value (NPV) of 53.3% and accuracy of 90%.The test had positive likelihood ratio of 8.11, negative likelihood ratio of 0.11 and diagnostic odds ratio of 1.82(95 % CI 0.75- 4.44).

Hernández-Garcés *et al.* (24) reported a positive predictive value of 89.8% and negative predictive value of 48.1% with diagnostic accuracy of 73.3%. The diagnostic odds ratio in their study was 8.17. Mitsuhashi *et al.* (21) reported positive predictive value and negative predictive value of 100% and 72.7%, respectively.

Our findings suggest that phenol red chromoendoscopy is a useful valid method for *H. pylori* detection with its advantages include immediate reading, ability to detect focal and scattered infection that can be missed by random biopsy taking and it also can be used when *H. pylori* invasive diagnosis is crucial and biopsy is contraindicated as in coagulation disorders.

Corresponding author:
Sameh Ahmed Abdel-bary
Internal Medicine Department, Faculty of Medicine,
Ain Shams University, Cairo, Egypt
Email address: samehmind@gmail.com

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