ORIGINAL PAPERS

Chromoendoscopy with red phenol in the diagnosis of *Helicobacter pylori* infection

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ABSTRACT

An analytic study to validate a diagnostic test was carried out at the Institute of Gastroenterology in Havana, Cuba in adult patients of both sexes in whom chromoendoscopy was carried out with red phenol at 0.1% over the gastric mucosa for the detection of *Helicobacter pylori* infection between November 2008 and December 2010. The staining with red phenol at 0.1% is included in the invasive tests for the diagnosis of *Helicobacter pylori* infection and of the reactive techniques. The sensibility of red phenol dye in the diagnosis of *Helicobacter pylori* infection in the patients studied was of 72.6% with a confidence interval (C.I.) of 95% (64.9 to 79.2%) and a specificity of 75.5% C.I. 95% (61.9 to 85.4%). The positive predictive value was of 89.8% C.I. 95% (83.1 to 94.1%) and the negative predictive value of 48.1% C.I. 95% (37.3 to 59.0%). The proportion of false positives was of 24.5% C.I. 95% (14.6 to 38.1%) and the proportion of false negatives was of 27.4% C.I. 95% (20.8 to 35.1%). The diagnostic accuracy of the dye on the patients studied was 73.3% C.I. 95% (66.7 to 79.0%). The diagnostic odds ratio was 8.17 C.I. 95% (3.88 to 17.23), the J Youden ratio of 0.5 and the Kappa coefficient of 0.40 C.I. 95% (0.27 to 0.54). The staining dye with red phenol at 0.1% resulted in a useful method in the diagnosis of *Helicobacter pylori* infection in the gastric mucosa, it can be applied in our environment and has multiple advantages (topographic localization, avoids contamination and fast and immediate reading).

Key words: Red phenol. Chromoendoscopy. *Helicobacter pylori*.

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INTRODUCTION

For the diagnosis of *Helicobacter pylori* (*H. pylori*) infection, we dispose of invasive tests like histology, cultures, molecular tests and rapid urease test which are dependent on the taking of biopsies from the gastric mucosa through an upper digestive endoscopy and of none invasive tests which do not require upper endoscopy like serology, urea breath test or the detection of stool antigens (1-3).

The rapid urease test is a simple, fast and cost-effective method in the detection of *H. pylori*. It is based on the urease enzyme's properties which contains the bacteria in high dosages and allows the urea to be metabolized and transformed into ammonium (3-5).

The staining of the gastrointestinal tract has received little attention from western investigators even though its widespread use in other parts of the world. However, the interest for this technique has grown in the last years thanks to it being simple, cheap, and fast and practically has no adverse effects. Also the application of new techniques of chromoendoscopy in the diagnosis and follow up of a variety of pathologies that increase its utility in the routine endoscopic practice (6-8).

A promising clinical utility of red phenol is for the endoscopic detection of *H. pylori* infection in the stomach, at least as a complementary technique to the ones that already exist. The bacterial urease produces hydrolysis of urea to ammoniac and carbon dioxide which would cause the increase in the pH and the red staining of the infected zones. Red phenol turns from yellow to red in a basic pH in the infected zones.

Because of its reactive and non-absorptive characteristic, it is rapidly eliminated from the organism through the digestive system, without a report of toxicity (9,10).

Presently, it is very important to validate methods, processes, etc., which allow higher confidence in results and that they show the aptitude for the use that they are destined to. All this motivated us to carry out the following investigation, with the objective to know the value of chromoendoscopy with

red phenol at 0.1% combined with the rapid urease test in the diagnosis of *H. pylori* infection of the gastric mucosa.

MATERIAL AND METHOD

An analytic study to validate a diagnostic test was carried out on all patients older than 18 years of both sexes who came to the endoscopy department at the Institute of Gastroenterology (I.G.E.) in Havana, Cuba, with an indication for upper digestive endoscopy for different reasons. To these, the chromoendoscopy technique with red phenol at 0.1% over gastric mucosa for the detection of *Helicobacter pylori* infection was applied in a period between November 2008 and December 2010.

The sample was constituted by 195 patients with a positive proportion estimated at 80, maximum accepted error of 0.08 and confidence interval of 95%.

All patients selected for this study fulfilled the following criteria.

Inclusion criteria

- Patients older than 18 years.
- Patients of both sexes who came to the department of digestive endoscopy at the I.G.E. with an indication for a diagnostic upper digestive endoscopy.
- Patients who with conformity and voluntarily participate in the study.

Exclusion criteria

- Patients below 18 years.
- Patients who are allergic to red phenol.
- Patients who do not wish to participate in the study.
- Patients who have received antisecretory treatment up to 6 months before the endoscopic study.
- Patients who have received treatment to eradicate *H. pylori* infection up to 6 months before the test.
- Patients with gastric surgery.

Exit criteria

- Insufficient sample of material from gastric biopsy or not useful for histological diagnosis.

Ethical considerations

The study is justified from an ethical point of view in accordance with the principles of the Helsinki declaration and without conflict of interest. Prior to the endoscopic study, a written consent was solicited from the patient to carry out the investigation, and the approval from the sci-

Fig. 1. Chromoendoscopic technique with red phenol at 0.1% over the gastric mucosa. Zone with red stain (positive staining) and zones with persisting yellow color (negative staining).

entific counsel of the institution was obtained, without conflict of interest.

Procedure

Upper digestive endoscopy was carried out on all patients by the same endoscopist, utilizing a GIF Olympus EVIS LUCERA 260 with frontal vision. Once the endoscopic diagnosis had been made, it was then proceeded to make the chromoendoscopic staining, using a spray type catheter (Olympus PW 5L-1) instilling 20 ml of red phenol at 0.1% over the mucosa of the gastric cavity in a homogeneous way, a minute was waited to visualize the reaction of the mucosa to the application of the dye. Subsequently, a sample of gastric mucosa was taken using a standard biopsy forceps (Olympus FB-25K-1) through the instrumentation channel from zones where there was reaction to the dye, red stain (positive), for histological study (gold standard), with the objective of identifying the presence or not of *H. pylori*. On patients where there wasn't a reactive staining of the dye, yellow color (negative staining), a biopsy sample of gastric mucosa from zones which are more frequently colonized by *H. pylori* (incisura angularis, prepyloric region and body), were taken for histological study with the objective of detecting or not the presence of *H. pylori* infection (Fig. 1). The samples were placed in a bottle with formol, which was labeled with the patient's data, date and type of sample; afterward, it was sent to the pathology laboratory for its histological study using hematoxylin-eosin and Giemsa without previous knowledge by the pathologist of the chromoendoscopic study.

The variables that were measured in our investigation were:

Variables

Age: It was considered according to completed years:

- From 18 to 30 years.
- From 31 to 40 years.
- From 41 to 50 years.
- From 51 to 60 years.
- Older than 60 years.

Fig. 2. Distribution of patients, positive or negative to *H. pylori* infection according to histological study. I.G.E. 2010.

Sex: According to biological sex.

- Feminine.
- Masculine.

Chromoendoscopic diagnosis

The following dye criteria were established for the reactive under validation (red phenol at 0.1%):

- Positive reactive staining: mucosa that takes a red color when put in contact with the dye.
- Negative reactive staining: mucosa takes a yellow color when put in contact with the dye.

Histological diagnosis

The histological diagnosis of the gastric mucosa was only limited to the morphologic description on the presence or not of *H. pylori* infection according to the Sydney System of one of the measurable variables (density of *H. pylori* infection).

The chromoendoscopic and histological diagnosis were related.

STATISTICAL ANALYSIS

An automated database was created using Excel 2007. Sex and age were analyzed in a general and independent way shown in totals and percentages. The evaluating indicators of a diagnostic test were determined comparing the test (dye with red phenol) with the gold standard (histological diagnosis). The following indicators were determined: sensitivity, specificity, positive predictive value, negative predictive value, proportion of false negatives, exactness in diagnosis, diagnostic odds ratio, J Youden ratio, probability quotient or verisimilitude ratio, pre-test probability.

The concordance between tests (red phenol and histology) was evaluated with the Kappa coefficient, with the

Fig. 3. Distribution of patients positive to *H. pylori* through histological study according to groups of ages and sex. I.G.E. 2010.

interpretation varying from -1 to 1. Data was processed using a Pentium 4 computer and Epiinfo 6.0.

RESULTS

As seen in figure 2 of the total patients under study, 146 patients (75.0%), had the presence of *H. pylori* infection demonstrated by histological study. When observing the distribution of patients with positive *H. pylori* confirmed by histology according to groups of age and sex (Fig. 3), we could observe that the majority of cases were in the group of over 60 years for females and 41 to 50 in males (26.7%).

Table I shows the statistical analysis from the relation between the chromoendoscopic diagnosis with red phenol at 0.1% and the histological diagnosis. Of 118 patients with a positive endoscopic staining, 106 had the presence of *H. pylori* infection through histological study. Of 77 patients with a negative endoscopic staining, the histological diagnosis was negative for *H. pylori* infection in 37 patients. According to these results, the sensibility of red phenol dye in the diagnosis of *H. pylori* in the gastric mucosa in patients studied was of 72.6% C.I. 95% (64.9 to 79.2%) and the specificity of 75.5% C.I. 95% (64.9 to 79.2%) and the specificity of 75.5% C.I. 95% (61.9 to 85.4%).

The positive predictive value of the test was of 89.8% C.I. 95% (83.1 to 94.1%) and the negative predictive value of 48.1% C.I. 95% (37.3 to 59.0%). The proportion of false positive was 24.5% C.I. 95% (14.6 to 38.1%) and the proportion of false negative of 27.4% C.I. 95% (20.8 to 35.1%). The exactitude in diagnosis of red phenol dye in the patients studied was of 73.3% C.I. 95% (66.7 to 79.0%).

The diagnostic odds ratio that has the advantage of gathering with a single indicator the exactitude of the test was

Table I. Relation between the chromoendoscopic diagnosis with red phenol at 0.1% and the histological diagnosis. I.G.E. 2010

Chromoendoscopic diagnosis	Histological diagnosis		Total
	Presence of <i>H. pylori</i>	Absence of <i>H. pylori</i>	
Red phenol at 0.1%. Positive reaction	106	12	118
Red phenol at 0.1%. Negative reaction	40	37	77
Total	146	49	195
C.I. 95%			
Sensibility	72.6%	64.9 to 79.2%	
Specificity	75.5%	61.9 to 85.4%	
Positive predictive value	89.8%	83.1 to 94.1%	
Negative predictive value	48.1%	37.3 to 59.0%	
Proportion of false positives	24.5%	14.6 to 38.1%	
Proportion of false negatives	27.4%	20.8 to 35.1%	
Exactitude	73.3%	66.7 to 79.0%	
Diagnostic odds ratio	8.17	3.88 to 17.23	
J Youden ratio	0.5		
pre-test probability (prevalence)	74.9%		

Source: database.

of 8.17 C.I. 95% (3.88 to 17.23). The J Youden ratio in our study, was 0.5 which the closer it is to 1, the higher the quality of the results obtained. Finally the Kappa coefficient (Table II) was of 0.40 C.I. 95% (0.27 to 0.54).

DISCUSSION

Helicobacter pylori is a bacteria that infects the gastric mucosa in more than 50% of the world population and it has been recognized as the most important etiologic factor for the development of diverse gastric affections like gastritis, gastroduodenal ulcer, gastric cancer and lymphoma (MALT). Because of the pathogenic potential of these bacteria, it has become necessary to count with efficient methods for its detection (11-13).

H. pylori infection is widely disseminated and its prevalence worldwide is 30.0 to 50.0% with variations all over the planet which in great measure depends on the global standard of living in each region. In developed countries, infection by this agent is infrequent in children but it increases gradually with age, in developing countries, most of its inhabitants are infected independent of age, these levels of infection reaching close to 70% (14,15).

Table II. Kappa coefficient

0,73	Total proportion of concordance observed
0,55	Expected proportion by random
C.I. 95%	
Índice Kappa	0,40
	0,27 to 0,54

Source: database.

Two studies carried out in Cuba at the Institute of Gastroenterology and "Faustino Perez" Hospital in Matanzas with series of more than 200 patients reported a prevalence of *H. pylori* infection of 80 and 78% respectively (16,17).

Results obtained during our study correspond with what is reported in the literature, 75% of patients evaluated had a histological confirmation of *H. pylori* infection.

Patients older than 60 years have a higher frequency of chronic atrophic gastritis and intestinal metaplasia; therefore the density of *H. pylori* decreases significantly (15,18,19).

In 2009 of a total of 2,783 upper digestive tract endoscopies done at the Institute of Gastroenterology in Cuba, 810 corresponded to patients older than 60 years, although this predominance, in our investigation the highest proportion of positive histological test for *H. pylori* were in the range between 41 to 50 years.

It has been described that *H. pylori* infection is cosmopolitan and affects both sexes equally. Gonzalez-Carvajal and other investigators express that sex doesn't seem to be an important question in the prevalence of infection in our country (16,17,20).

At the end of our study, we observed predominance in the female sex, however we consider that this difference is due to the casuistic way of selecting patients and because the female sex come more often to the endoscopy department. In the year 2009 of a total of 2,783 upper digestive tract endoscopies done, 1,571 were on females (56.4%).

The chromoendoscopy techniques are alternatives to reduce diagnostic errors according to the Japanese society in its recent revision and consensus (2009-2010), levels of sensibility and specificity were established for the different diagnostic methods of *H. pylori* infection, which should be applied by the endoscopic services in an individualized way according to the socio-economic conditions and most frequent pathologies (21,22).

The rapid urease test has good sensibility and specificity, 83 to 74.3% respectively. Other authors, like Malferttheiner and collaborators or in the Maastricht III (2007) consensus, report superior results which places it closer to the gold standard which is the histological study in any of its variants (sensibility of 90-93% and specificity of 95-99%) (23,24).

Due to its simplicity, low cost and its fast, the urease test is considered the technique of election for the initial diagnosis of *H. pylori* infection in those patients that have an endoscopy done (25).

The cost of applying red phenol over the gastric mucosa does not differ from that of the urease test and has as advantage that it allows the survey of the mucosa so as to direct the taking of samples, it avoids false positives and/or negatives related with the contamination due to manipulation of samples, temperature, conservation and reading of the same (26-29).

The sensibility, specificity and exactitude in diagnosis with red phenol at 0.1% in our medium was over 70.0%, this allows us to affirm that this method is useful in the detection of *H. pylori* infection of the gastric mucosa. When calculating the diagnostic odds ratio, the utility of the test was shown. Patients with false negative results had a low density of *H. pylori* infection of the gastric mucosa and on the endoscopic findings, eritematose-erosive gastritis predominated. Most patients with gastroduodenal ulcer or erosive duodenitis were positive to red phenol dye, corroborated with histological study.

The J Youden ratio had an intermediate value and when evaluating the grade of concordance between the two diagnostic tests (Kappa coefficient), in our investigation the test had a moderate value in the diagnosis of *H. pylori* infection. Because there was only one observer, the grade of interobserver concordance could not be evaluated, but we consider that it is not a difficulty in the validation test because only the change of color from yellow to red was evaluated which is not confusing and simple to analyze.

In a study done by Dr. Juan Manuel Ahumada and collaborators in Mexico with 160 patients, they correlated the coloring with red phenol and histology obtaining values of sensibility 91.0%, specificity of 89.0% and Kappa coefficient of 0.73. Cho and collaborators in Korea report similar results (sensibility 95%, specificity 93% and Kappa coefficient 0.80), in this investigation they correlated red phenol with breath test with C¹³, including 200 patients. Lastly a Japanese study carried out by Mitsuhashi and Mitomi, correlating the dye with red phenol and culture with the variation of using it in patients with gastric surgery (178 patients), the sensibility and specificity was of 81.0% and 79.0% respectively with a Kappa coefficient of 0.59 (10,30).

In our investigation, staining with red phenol at 0.1% resulted in a useful method in the diagnosis of *H. pylori* infection of the gastric mucosa and can be applied in our environment, also counting with multiple advantages (topographic location, it avoids contamination, fast and immediate reading, etc.), and can be used in patients with contraindications for the taking of biopsy samples (coagulation disorders).

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