Comparison of helicobacter serum

Abdelrahman E. Mohamed, FRCP, Mohamed A. Al-Karawi, Facharzt,
Roger H. Higham, FIBMS, Abimola O. Osoba, FRCPath (UK),
Zuhal Ghandour, MD, Mohamed I. Yasawy, MD.
Mohamed H. Gaii, MRCP, Asad U. Qureshi, DC (Lond)

ABSTRACT

Objectives: The objective of the study is to compare the helicobacter pylori high molecular weight cell associated protein, IgG enzyme immunoassay (HM.CAP, IgG EIA) with the campylobacter-like organism preformed urease campylobacter-like-organism (CLO test) as an indication of the presence of H. pylori among patients with dyspepsia.

Subjects: One hundred and six Saudi patients with dyspepsia or abdominal pain who had gastroscopy between July and September 1993, at the Gastroenterology Department of the Riyadh Armed Forces Hospital, were included in the study.

Design: Antral biopsy was collected from each patient for CLO test, while a serum sample was obtained for H. pylori immunoassay test using the HM.CAP immunoassay kit from Enteric Products Inc. Specimens were sent to the microbiology laboratory blind without indicating the clinical findings or the result of the CLO test performed in the Gastroenterology Department.

Results: In 60 of these patients (56.6%) both tests were positive and in 12 cases both tests were negative. The concordance of the two tests was 68%. The most common abnormal gastroscopic findings were gastritis or gastric erosion in 24 patients and in 12 (50%) of them, both the CLO test and serology were positive.

Conclusions: Both tests were found useful for screening for the presence of H. pylori. A screening strategy based on serology could play an important role in reducing endoscopy workload and for follow-up of patients with H. pylori infection.

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Keywords: Helicobacter pylori, IgG EIA serology, CLO test, dyspepsia.

Helicobacter pylori has been implicated as a major cause of type B gastritis and peptic ulcer disease. In addition, it has also been linked with gastric cancer in some reports. Identification of H. pylori is usually made by histological examination of biopsy material, isolation of the organism on selective culture media or tissue assay for the presence of urease in samples of gastric material. All these methods require biopsy of the gastric tissue and are often subject to sampling error and the relative risk of an invasive procedure. Consequently, attention has been directed to non-invasive methods of diagnosis. The first of these tests is the urease dependent breath test. This is a simple technique but is labor-intensive and expensive. The test most frequently used in endoscopy units is the commercially available CLO test (campylobacter-like-organism). With this test, biopsy samples are tested for the presence of urease activity. The CLO test detects only preformed urease activity, while further urease production by H. pylori or other contaminating bacteria is prevented by the bacterio-static agent in the gel. Reports of sensitivities and specificities ranged from 65-95% and 60-100% respectively. More recently there has been increased interest in the use of non-invasive techniques for the diagnosis and follow-up of patients treated for H. pylori infection. Measurement of H. pylori IgG serum antibodies by means of enzyme-linked immunosorbent assay (EIA) has been reported to be a reliable, inexpensive and non-invasive method for the detection of infection, for the evaluation of the effect of eradication therapy and for epidemiological studies. Higher antibody concentrations were found to reflect a more active gastritis and a higher prevalence of duodenal ulceration. The main aim of this study is to compare the H. pylori high molecular weight cell associated protein, IgG enzyme immunoassay (HM.CAP IgG, EIA) with the campylobacter-like organism preformed urease (CLO test) as an indication of H. pylori infection among patients.

From the Department of Gastroenterology (Mohamed, Al-Karawi, Ghandour, Yasawy, Gaii) and Microbiology (Higham, Osoba, Qureshi), Armed Forces Hospital, Riyadh.

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Address correspondence and reprint request to: Dr. A.E. Mohamed (C149), Department of Gastroenterology, Armed Forces Hospital, PO Box 7897, Riyadh 11159, Saudi Arabia.
with dyspepsia.

**Materials and methods** One hundred and six Saudi patients with dyspepsia or abdominal pain who had gastroscopy at the Gastroenterology Department of the Riyadh Armed Forces Hospital between July and September 1993, were included in the study. None of these patients was diagnosed as having *H. pylori* infection before, nor had received any treatment for *H. pylori* before this study. In each patient, the gastroscopic findings were recorded and one endoscopic biopsy was taken from the antrum. The biopsy specimen was placed in the CLO test (Delta West Ltd., Canning Vale, Western Australia) which was performed in the endoscopic department and result checked every half hour up to 6 hours and recorded. At the same time, 10 ml of clotted blood in a sterile container was collected and sent to the microbiology laboratory for the *H. pylori* immunoassay test using the HM.CAP immunoassay kit from Enteric Products Inc. (Westbury, NY). Serum samples were stored at 20 °C until the test was ready to be performed. The methodology used was that recommended by the manufacturers. The kit detects *H. pylori* IgG anti-bodies in about 60 minutes using a purified high molecular weight cell associated protein-CAP antigen.14,15 The specimens were sent to the laboratory blind without indicating the clinical findings or the CLO test result. All tests were carried out in duplicate. The HM.CAP is based on standard EIA protocols with the added benefit of stable liquid ready-to-use reagents. Patients serum samples to be assayed for antibody were first diluted and incubated with the purified *H. pylori* antigen bound to the solid surface of the microwell. Diluted patient sera or standards were added to wells and incubated at 19-26 °C for 20 minutes, washed 3 times and 0.100 ml of substrate solution was added. Incubated at 19-26 °C for 10 minutes, acid stop solution was added; absorbance measured at a primary wavelength of 450 nm. Negative, high and low calibrators were included in each run. Absorbencies were converted into values (EV) for each sample. Values >2.2 were considered positive and less than 1.8 considered negative. Values between 1.8 and 2.2 were considered as borderline and repeated.

**Results** The 106 patients assessed prospectively were aged 16 to 66 years. Seventy-nine of these patients were between 25 to 50 years and the remaining 27 patients were below 25 years (11 patients) or above 50 years (16 patients). A positive CLO test was recorded when a color change to pink was observed. The result was recorded either as positive or negative. The HM.CAP EIA results were read as stated above. In Table 1, the patients have been categorized according to the gastroscopic findings. In 60 patients, both tests were positive and in 12 cases, both tests were negative. However, 18 patients were negative by the HM.CAP EIA test while the CLO test was positive. Similarly, in 16 cases, the HM.CAP EIA was positive while the CLO test was negative (Table 1). The concordance of the two tests was 68%. Among 38 patients with normal gastroscopic findings 22 (58%) were positive by both serology and CLO test. The serological test detected 9 additional patients with significant *H. pylori* IgG antibodies. The most common abnormal gastroscopic finding was gastric or gastric erosion in 24 patients and in 12 (50%) of them, both the CLO test and serology were positive. In 9 other patients, either the CLO test or serology was positive. Sixty patients had both positive serology as well as CLO test, including the 27 patients who were below 25 years of age or above 50 years of age.

**Discussion** This study shows that there is a high prevalence of *H. pylori* infection and/or

<table>
<thead>
<tr>
<th>Gastroscopic diagnosis</th>
<th>CLO test positive; Serology positive</th>
<th>CLO test positive; Serology negative</th>
<th>CLO test negative; Serology positive</th>
<th>CLO test negative; Serology negative</th>
<th>Total no of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>22</td>
<td>1</td>
<td>9</td>
<td>6</td>
<td>38</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Gastritis and erosion</td>
<td>12</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>Duodenitis</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Hiatus hernia and GERD*</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Other **</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>TOTAL</td>
<td>60</td>
<td>18</td>
<td>16</td>
<td>12</td>
<td>106</td>
</tr>
</tbody>
</table>

* GERD: Gastroesophageal reflux disease.
** Other: Gastric tumor (2); Bile reflux (2); Esophageal varices (3); Gastronemunostomy (1)
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colonyization among Saudi patients as 94 (88.7%) out of 106 patients had either a positive CLO test or serology or both. Considering the endoscopic findings, 22 patients (21%) out of 106 were regarded as normal but were positive for both tests. It is probable that these patients were colonized and had not developed any lesions (Table 1). The HM.CAP EIA detected an additional 9 patients with normal gastroscopic findings and positive serology. These patients will need to be followed up to see if they subsequently develop additional signs and symptoms. Both tests were positive in 21 patients with duodenal ulcer, gastritis with erosion and duodenitis, all these conditions are associated with H pylori infection. The CLO test detected an additional 13 patients in this group while the HM.CAP EIA was negative, probably due to failure of these patients to mount a detectable level of IgG antibodies. On the other hand, the HM.CAP EIA detected 6 patients where the CLO test was negative which could be regarded as false negative tests. In the 4 patients with gastric ulcer both tests were positive as expected in view of the association of H pylori with gastric ulcer. From our previous study no single test, including histology, will identify all cases of H pylori infection and colonization, although histology is still the most superior of all tests available. The gold standard for the diagnosis of H pylori has been defined as either a positive culture or positive histological identification of the organism. However, it is to be remembered that the distribution of H pylori in the gastric mucosa is patchy and the predictive values of culture and histology depend upon the numbers of samples obtained. Multiple samples are frequently required to avoid sampling errors. From this previous study of 196 patients, H pylori was identified by histopathology in 145 patients (73.98%), the CLO test was positive in 126 patients (64.29%) and a positive culture was obtained in 102 patients (52.04%). There are now several reports from various provinces in the Kingdom of Saudi Arabia on the prevalence of helicobacter infection, describing the association of H pylori with various disease processes. In many of these reports, diagnosis of H pylori infection is usually carried out by histological examination of a biopsy sample as well as a urease test such as the CLO test or a similar test. The handicap of these tests are that they require the time and expertise of an endoscopist and they are invasive.

In most endoscopy departments the demand for endoscopy exceeds the resources and facilities available. Endoscopy for all patients presenting with dyspepsia takes considerable time and effort. The use of helicobacter EIA test such as HM.CAP offers several attractive advantages. Firstly, it allows for screening of young patients presenting with dyspepsia and debilitated patients for H pylori infection. In our study 11 patients who were below 25 years of age had both positive serology and CLO test. Secondly, patients seen in primary care centers, district hospitals, etc., where there are no endoscopists, can be screened, to select patients for endoscopy referral. Thirdly, IgG titres have been found to decrease sharply and rapidly following adequate therapy for H pylori infection. Hence helicobacter EIA could be used for follow-up to determine the success of therapy and predict or confirm a relapse of infection. Recently many studies have been performed to investigate the mode of transmission and incidence of H pylori infections in various population groups.

H pylori EIA offers itself as an epidemiological tool which is inexpensive and non-invasive and can be performed in any laboratory performing EIA assays. In a recent study comparing endoscopic biopsy with helicobacter EIA (in which infection is defined by either a positive culture or histological staining), HM.CAP EIA was reported to be sensitive and specific for detection of active disease. The authors conclude that serologic testing has proved so successful in identifying ongoing infection that they have suggested that serological analysis rather than biopsy be considered the gold standard for diagnosis of H pylori infection. Colonization with H pylori produces an immune response and specific IgM, IgG & IgA antibodies are detectable using enzyme-linked immunosorbant assays. The HM.CAP kit used in this study utilizes a high molecular weight cell-associated protein. HM.CAP antigen, (to detect H pylori IgG), which has been reported to give excellent specificity and sensitivity, i.e. 100% and 98.7% respectively with no cross-reaction with campylobacter jejuni, C fetus and Escherichia coli. The HM.CAP test is a qualitative test and blood samples obtained too early during infection may not contain detectable antibodies as found in 13 patients in this study. On the other hand, false-negative CLO test has been reported to arise either from low bacterial density or from sampling error. In cases of extensive intestinal metaplasia or of patchy distribution of the organisms on the mucosa. We have found 6 patients who have fallen into this category. Nevertheless, both tests are useful for screening for the presence of H pylori. In 12 patients both CLO test and serology were negative. Histological examination of antral biopsy is recommended as gold standard but this was not carried out in all our patients, as this study was only a comparison between CLO test and serology and we have studied other different diagnostic methods before. However, histology examination with H and E, was not carried out in these 12 patients (as this was not included in the study) and histology examination might have been positive in some of these patients.

Al Muagel et al studied by serological tests

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the prevalence of *H. pylori* in Riyadh Region, and found that the prevalence of *H. pylori* infection increased rapidly with age. More than 70% of those 20 years or older were positive. Lin et al studied different diagnostic tests to determine *H. pylori* infection and found that the sensitivity and specificity of serology was 96% and 88%, respectively.

**Conclusion**

Our findings support that of Sobala et al that screening strategy based on *H. pylori* serological status and age and further clinical details, have an important role in reducing endoscopy workload. However, in symptomatic patients, failed therapy or relapse, the value of endoscopy remains undiminished.

**References**