Evaluation of a commercial enzyme-linked immunosorbent assay (ELISA) kit for serological diagnosis of *Helicobacter pylori* infection in a group of non-ulcer dyspepsia sufferers

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Summary: *Helicobacter pylori* infection of the stomach is associated with gastritis and peptic ulceration and may be causative. A noninvasive test for this organism might be useful in managing some patients with dyspepsia without the need for further investigation. We have evaluated a new commercially available serological test (Helico-G™ ELISA, Porton Cambridge, UK) for this infection to assess its diagnostic accuracy in a retrospective study of 115 patients with nonulcer dyspepsia. Sixty-three of these patients (55%) were found to have *H. pylori* infection and gastritis on histology. A sensitivity of 81% and specificity of 90% were obtained. No significant fall in the antibody titres was found in a subgroup of 15 patients who were selected to complete a course of triple therapy despite significant improvement in their dyspepsia score and confirmed eradication of *H. pylori* organism in 80% of these patients. We conclude that the test has limited value in aiding clinical decision of managing patients with dyspepsia.

Introduction

The recent 'discovery' of *Helicobacter pylori* in association with peptic ulcer disease and non-ulcer dyspepsia^{1–3} has generated tremendous impetus in research at clinical as well as laboratory levels. The interest in this organism has largely been rekindled and pioneered by Warren and Marshall^{4,5} who demonstrated that this 'century old' microaerophilic spiral Gram-negative bacillus could account for chronic active gastritis as well as peptic ulcer disease. Their claim has since been substantiated by human volunteer and animals studies.^{6–9} A number of methods are currently available for detection of *H. pylori* infection¹⁰ (also see review²). Although the noninvasive tests (urea breath tests and enzyme-linked immunosorbent assay (ELISA)) have recently received considerable attention, there are those who still prefer the gold standard tests of histology and microbiology² to diagnose *H. pylori* infection. The urea breath tests are promising in diagnosis and follow-up but disadvantages are the requirement for a costly mass spectrometer and the potential danger with recurrent exposure to radioactivity.

Reports on the usefulness of ELISA as an alternative noninvasive diagnostic and screening

test for *H. pylori* infection are encouraging.^{11–13} The majority of assays have employed bacterial extracts as antigens for detecting IgG antibody and show high sensitivity and specificity for diagnosis. Newell *et al.*¹² developed the Helico-G™ ELISA and claimed sensitivity of 95.6% and specificity of 83% for their assay which has recently been marketed for aiding serological diagnosis of *H. pylori*. The same assay yielded sensitivity between 82 and 97% and specificity 87–100% in two recent studies.^{15,19} In this study, we aimed to evaluate the accuracy of this commercially available ELISA for serological diagnosis of *H. pylori* and compared the results with the conventional tests, histology and urea broth test.

Materials and methods

Confirmation of antral gastritis and H. pylori infection

A retrospective analysis of 115 patients (46 males and 69 females; mean age of 38.5 years, range 19–53 years) with a final diagnosis of non-ulcer dyspepsia was performed in this study. None of these patients had received treatment other than antacids within 3 months of their endoscopy. *H. pylori* was detected by cytological brushing urea broth test and histology as described before.²⁰ The

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presence of gastritis and the number of *H. pylori* organisms were similarly assessed as described previously.²⁰ Serum was obtained from each patient and stored at -20°C until required. Fifteen *H. pylori*-infected patients who had significant dyspepsia and elevated Helico-G titres were given a course of tripotassium dicitrato bismuthate (120 mg four times daily) for 4 weeks, amoxycillin (500 mg three times daily) and metronidazole (400 mg three times daily) for the first 10 days in an attempt to eradicate the organism. The dyspepsia scores, arbitrarily graded from 0 to 3 (0 = asymptomatic; 1 = mild dyspepsia; 2 = moderate dyspepsia; and 3 = severe dyspepsia) were recorded before and after treatment. Histology, cytological brushing urea broth test and the Helico-G titres were re-evaluated after 12 weeks.

Enzyme-linked immunosorbent assay (ELISA)

Helico-GTM ELISA is a commercially available test (Porton Cambridge, UK) for serological diagnosis of *H. pylori*. It contains micro-ELISA wells coated with acid glycine extracts of *H. pylori* surface proteins. It provides a quantitative analysis of the anti-*H. pylori* antibody titre that is present in patients' serum. The assay was performed on all the sera collected according to the manufacturer's instructions. The final results of the tested sera were expressed as U/ml Helico-G titres with reference to the standards provided. The intraplate and interplate variation was assessed to check reproducibility of the test by repeating analysis of three *H. pylori*-positive and three *H. pylori*-negative patients' sera.

Statistics

Mann-Whitney *U* test was used to assess the Helico-G antibody titres between the *H. pylori* positive and the *H. pylori* negative groups, the Helico-G titres and the number of *H. pylori* organisms present histologically in the *H. pylori* positive group. Furthermore it was also used to compare the dyspepsia scores and the Helico-G titres difference before and after triple medical therapy in the 15 selected patients. $P < 0.05$ was accepted as a statistically significant result.

Results

Sixty-three out of the 115 (55%) patients studied were found to be *H. pylori* positive by both the histology and cytological brushing urea broth test. Chronic atrophic gastritis with the presence of increased number of plasma cells and polymorphs infiltration was observed in all the 63 (100%) *H. pylori*-positive patients. The remaining 52

patients showed negative cytological brushing urea broth test and normal histology without inflammation or *H. pylori* organism.

The upper normal range of Helico-G titre was designated as 10 U/ml as recommended by the company. Elevated Helico-G titres were found in 51/63 *H. pylori*-positive (sensitivity 80.95%) and 5/52 *H. pylori*-negative (specificity 90.38%) patients (Figure 1 and Table I). The median Helico-G titre was 31.2 U/ml and 5.8 U/ml in the *H. pylori*-positive and the *H. pylori*-negative groups, respectively. The *H. pylori*-positive group had significantly higher Helico-G titres than the *H. pylori*-negative group ($P < 0.0001$). There was no correlation between the Helico-G titres and the number of *H. pylori* organisms present ($P > 0.05$). The mean ($\pm$ SEM) intraplate and interplate variation of the Helico-G ELISA was found to be 9.2% ($\pm$ 3.3) and 14.8% ($\pm$ 3.8), respectively.

Eleven out of the 15 (73%) treated patients showed significant improvement in their mean dyspepsia score from 2.7 before treatment to 1.2 after treatment ($P < 0.01$). Three out of these 15

Figure 1 Scattergram of the Helico-G ELISA results (NR = normal range; $\blacksquare$ and $\bullet$ = median Helico-G titres in the *H. pylori*-negative and *H. pylori*-positive group, respectively).

Table I The results of the Helico-G ELISA

Sensitivity	81%
Specificity	90%
Positive predictive value	91%
Negative predictive value	80%
Efficiency	85%

(20%) patients remained *H. pylori* positive to cytological brushing urea broth test after triple medical therapy. These three patients demonstrated unchanged histological appearance of chronic atrophic gastritis with *H. pylori* scattered on the surface epithelium. The other 12 patients showed virtually normal gastric epithelium with minimal scattered submucosal fibrosis. *H. pylori* was not seen in these follow-up biopsy samples. Two out of three unresponded patients had largely unchanged Helico-G titres whereas the third demonstrated further elevation of the Helico-G titres (Figure 2). Only one out of the 12 patients who had eradication of *H. pylori* showed reduction of the Helico-G titre to the normal range (Figure 2). Overall, there was no significant difference in the Helico-G titres before and after triple therapy ($P > 0.05$).

Figure 2 The Helico-G titres before and after triple medical therapy in the 15 selected patients.

Discussion

This study has shown that the commercially available Helico-G™ ELISA test has a reasonable sensitivity (81%) and specificity (90%) for detecting *H. pylori* infection serologically when the upper normal range is preset at 10 U/ml as recommended. However, the sensitivity falls short of the original report by Newell *et al.*¹² and the company's quoted figure of 95%. By using the recommended upper normal limit (10 U/ml) Logan *et al.*¹⁹ reported sensitivity (82%) very similar to our current study. Sobala and colleagues¹⁵ reported a much higher sensitivity of 97%. However, this was achieved by lowering the upper normal limit to 7.7 U/ml. Even if the upper normal range were to be adjusted to yield specificity of 83% to match the original report¹² the sensitivity would merely improve to 84% in this study. Therefore, like Logan *et al.*¹⁹ we were unable to reproduce the high sensitivity claimed by the company. Nevertheless, the test showed acceptable mean intraplate and interplate variation of 9% and 15%, respectively.

ELISA for *H. pylori* infection has been advocated for use as a screening test in young dyspeptic patients who have no suspicion of underlying upper gastrointestinal tract malignancy. It was claimed that about one in four endoscopies might be avoided if the assay had been used as a screening test.^{14,15} However, the number of patients studied was small. Further prospective evaluation is required to support its role as a reliable screening tool before it is introduced into clinical practice.

In our study, the false negative rate was approximately 19% when 10 U/ml of Helico-G titre was chosen as the upper normal limit. Why we encountered such a high percentage of false negative results is unclear. It may be due either to the failure to seroconvert or poor antibody response in patients who have scanty bacterial colonization of the stomach as suggested previously.¹⁰ However, this was not borne out in this study as there was no correlation found between the number of *H. pylori* organisms present histologically and the Helico-G titres. In order to adopt ELISA as a screening tool for epidemiological surveys a much higher sensitivity is essential, approaching the results reported by Evans *et al.* (sensitivity 98.7% and specificity 100%).¹⁷ Otherwise, it would mean significant underestimation of the true prevalence of *H. pylori*-positive cases. The false positive rate in this study was approximately 10% which also is unacceptable if accurate epidemiological data is required.

Apart from its diagnostic and screening potential the ELISA for *H. pylori* infection has been claimed useful in monitoring response to medical therapy and predicting relapse.^{13,21-23} Our experience in a small subgroup of 15 patients who had

received triple therapy and had antibody titres re-estimated after 12 weeks did not support such claims. Only a small proportion of these patients showed significant fall in Helico-G titres despite significant improvement in their symptoms, confirmed eradication of *H. pylori* and improvement in gastritis in the majority. The failure to demonstrate significant reduction in antibody titres in this study is unlikely to be attributed to the inadequacy of the Helico-G test. It may suggest a longer follow-up period is required to show the improvement. An extended study is underway to evaluate its role in monitoring patients' progress after combination therapy.

The link between *H. pylori* and duodenal ulcer disease is now widely accepted. However, the relationship between *H. pylori* and non-ulcer dyspepsia which has a prevalence range from 39% to 87%^{24,25} remains controversial. *H. pylori* infection has been found to be present in 55% of the patients with symptoms compatible with the diagnosis of non-ulcer dyspepsia. In this study, the presence of *H. pylori* correlated well with histo-

logical gastritis which were both eliminated after successful treatment with triple therapy. This lends further support to the causal relationship between *H. pylori* infection and gastritis.

In conclusion, the Helico-G ELISA test lacks accuracy in our study. Furthermore, the results are disappointing when it is used to monitor patients' progress after attempted eradication therapy. However, the result may be very different if a longer follow-up period is allowed. Finally, gastritis due to *H. pylori* infection accounts for more than half of the final diagnosis in patients with non-ulcer dyspepsia. Triple medical therapy not only helps to eradicate *H. pylori* and improve histological gastritis but also improves patients' symptomatology. We feel that cytological brushing urea broth test and/or histological examination of gastric biopsies for the presence of gastritis and *H. pylori* infection should be carried out in patients with non-ulcer dyspepsia so that a positive diagnosis could be made and an appropriate remedy may be prescribed to help improve the patients' quality of life.

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