

COMPARISON OF CARBON-14-UREA BREATH TEST AND RAPID UREASE TEST WITH GASTRIC BIOPSY FOR IDENTIFICATION OF *HELICOBACTER PYLORI*

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It is well known that *Helicobacter pylori* (*H. pylori*) infection of the gastroduodenal mucosa causes chronic active gastritis and may dispose to peptic ulcer disease. Several invasive and noninvasive methods to detect *H. pylori* infection of the stomach have been described in the literature. Until recently, the practice has mainly been focused on the detection of *H. pylori* by endoscopic biopsy of the gastric mucosa, and documentation of its presence, either by histological identification of *H. pylori* or culture of antral biopsies. We know that *H. pylori* produces large amounts of urease, which buffer surrounding hydrogen ions by the production of ammonia and bicarbonate.¹ The production of urease by *H. pylori* has been used in recent years to detect its infection of the gastric mucosa.

Graham et al. were the first to report the use of a breath test for the detection of gastric urease activity.² Recent modifications have included the use of carbon-14-labeled urea, the use of lower doses of radio-labeled urea, and corrections for the rates of carbon dioxide (CO₂) production based on the patient's weight.³

The purpose of our study was to compare the newly instituted noninvasive C-14-urea breath test (UBT) in our institution and the bedside urease test (CLO-TEST, manufactured by Delta West Pty Ltd, Bentley, Western Australia), with histological staining results for *H. pylori* in gastric biopsies. CLO-TEST is a cheap and easily used urease test performed on the antral biopsy. The test depends on the urease enzyme that produces a pH change in an indicator system. The UBT also uses the urease enzyme as an indicator for infection of the stomach with *H. pylori*.⁴

Patients and Methods

Sixty-four male and female patients aged between 18 and 79 years, with upper gastrointestinal symptoms, and referred for endoscopy at King Faisal Specialist Hospital and Research Centre, were prospectively studied.

All patients had at least two biopsy specimens from the gastric antrum. Patients with severe reflux esophagitis, severe gastritis, duodenal ulcer, gastric ulcer or malignancy were excluded from the study. Only patients with mild gastritis, duodenitis or esophagitis were included, and these were put on antacids. Staining of the gastric biopsies to detect *H. pylori* was done by using hematoxylin-eosin, alcian blue and PAS. All tissue sections were reviewed by an experienced pathologist for the simple presence or absence of *H. pylori*.

The results of the sixty-four biopsies for *H. pylori* were compared with the results of the other two methods, CLO-TEST and UBT. The UBT was performed after we ensured that the patients were not on antibiotics, omeprazole or bismuth preparations for the weeks between the endoscopy and the UBT. Patients were also asked to discontinue all other drugs, including antacids, for at least 12 hours prior to the test, if possible. For UBT, the patients were asked to fast overnight. For studies in the afternoon, patients were allowed a light breakfast and then were asked to fast for a minimum of four hours. Water was allowed as desired. CLO-TEST tests were performed at the time of endoscopy and biopsy, according to the manufacturer's instructions (Delta West, Bentley, Australia).

UBT was performed within two weeks of the endoscopy without intervening antibiotic therapy. The UBT was performed in the method previously described elsewhere.³ C-14-urea (Amersham, U.K.) was received freeze dried, reconstituted with sterile water, aliquoted into single-patient doses and stored frozen, as in a previously described method.⁵ All breath samples were collected using a simple disposable system, whereby a piece of plastic tubing was used to bubble the patient's breath through a trapping solution in a scintillation vial. Each vial contained 4 mL of 0.5 M benzethonium hydroxide (hyamine), with a trace of thymolphthalein indicator. Trapping of 2 mmol of CO₂ for each sample was indicated by the color change from blue to clear. A baseline breath sample was acquired to determine background counts. Patients were asked to rinse out their mouth with water and drink 0.111 MBq of C-14-urea in 25 mL of distilled water. Patients were then asked to rinse out their mouth twice with water without swallowing. Patients were kept sitting for exactly 20 minutes, and were again asked to rinse out their mouth with water, after which an additional breath sample was acquired.

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Accepted for publication 20 January 2000. Received 28 June 1999.

A standard solution containing 5% of the administered dose of C-14-urea (5.55×10^{-3} MBq) in 4 mL of distilled water in a scintillation vial was added to each set of samples. All samples were counted in a Beta counter with quench correction after the addition of 4 mL of scintillation fluid (Optiphase-Wallac, Finland). The percentage of administered dose in the sample at 20 minutes was calculated, and results were expressed as the CO₂ recovery in the 20-minute sample, as previously described,³ using the following formula:

$$\text{CO}_2 \text{ recovery} = \frac{(\% \text{ administered dose in sample}) \times (\text{weight in kg})}{2 \text{ mmol CO}_2 \text{ trapped}}$$

A patient was considered infected with *H. pylori* if the CO₂ recovery at 20 minutes was over 0.55, based on previously published results with a virtually identical technique.³ Values under 0.55 were considered as noninfected.

Results

We used the gastric antral biopsy as the reference value (gold standard) for comparison with the two other methods. Of the 64 gastric biopsies, 34 were positive for *H. pylori*, and 30 were negative. Of the 34 patients positive on histopathology, 30 were positive in the CLO-TEST and 29 patients were positive in the UBT. Of the 30 patients with negative histopathology for *H. pylori*, 26 were negative in the CLO-TEST and 21 were negative in the UBT.

Using the histopathology as the gold standard, the CLO-TEST had an 88% sensitivity and 87% specificity, with an overall accuracy of 88%. The results of the UBT showed 85% sensitivity and 70% specificity, and an overall accuracy of 78% (Table 1).

Discussion

There have been a variety of publications describing the results of various methods of diagnosing *H. pylori* infection.^{2,3,5-9} The most noninvasive methodology is clearly the urea breath test, using either carbon-13^{2,9} or carbon-14^{3,5-8} as the tracer label. Carbon-13-UBT testing requires specialized equipment, and is much more expensive than the carbon-14-UBT. Although C-14-urea is a radioactive material and does involve a radiation dose to the patient, the actual radiation doses delivered are very small and acceptable, even for the repetitive testing that may be necessary in cases of treatment-resistant or recurrent infections.¹⁰

TABLE 1. Using histopathology as gold standard, reliability of CLO-TEST and carbon-14 urea breath test are as follows.

| | CLO-TEST | C-14 UBT |
|---------------------------|----------|----------|
| Sensitivity test | 88% | 85% |
| Specificity | 87% | 70% |
| Positive predictive value | 88% | 76% |
| Negative predictive value | 87% | 81% |
| Overall accuracy | 88% | 78% |

A number of studies have confirmed the very high sensitivity and specificity of UBT for both initial diagnosis and documentation of successful eradication therapy.^{3,5-8} In most studies, the sensitivities reported have averaged between 91%-98%, with specificities between 84%-100%.^{3,5-8} Our sensitivity of only 85% is somewhat lower. This may have been due to separations of up to two weeks as a result of limited schedule slots between obtaining the biopsy and the breath test, or perhaps to the use of a CO₂ recovery cutoff value of 0.55.

Since setting up our assay, Desroches et al. have reported a cutoff value of only 0.33 for virtually the same methodology.⁶ Indeed, we currently consider a value between 0.35 and 0.55 as an indeterminate or borderline value, although fortunately, very few results fall into this range. However, reanalysis of our data using a cutoff of 0.33 only altered the classification of one patient, still leaving our sensitivity at less than 90%, although equal to the CLO-TEST. The actual reasons for our sensitivity being below 90% remain unclear, although perhaps analysis of data from a larger number of patients studied at the same time as endoscopy might improve the sensitivity.

The specificity of the breath test in our study was also lower than in other series, but in many patients, our failure to analyze histology from more than one biopsy may have resulted in sampling errors. This potentially incomplete pathological examination may have resulted in some patients with *H. pylori* infection being missed, resulting in an inappropriately high number of false positives on the UBT, thus falsely lowering specificity. Since the study of this series, the standard practice at our hospital has been to try to acquire four separate antral biopsies to reduce the possibility of inadequate pathological sampling. Other data might suggest that the pathological examination in our series underestimated the number of truly infected patients. Our finding of positive *H. pylori* pathology in only 53% of our patients is lower than the 64%-88.5% incidence reported in several other studies of *H. pylori* infection in symptomatic patients over 16 years of age in the neighboring countries of Jordan and Kuwait.^{11,12}

C-14-urea breath testing offers many advantages over other techniques. Not only is it noninvasive and inexpensive (we estimate the total cost of the test, including labor and materials, at 50-75 Saudi riyals, approximately \$13-20), but it is well tolerated by patients and easy for almost any patient to take. The total time for a patient to undergo the test is approximately 30 minutes, and one technologist can perform up to 5-6 tests per hour, using staggered appointments, thus causing minimal disruption of even a busy nuclear medicine department. Any hospital with a simple beta counter can perform this study. It has become our standard for the detection and follow-up of *H. pylori* infections in most patients.

In conclusion, C-14-urea breath testing is a safe, simple, inexpensive method of accurately detecting *H. pylori* infection, yielding results which are comparable to those obtained after using a CLO-TEST test at endoscopy and actual pathological examination of biopsy specimens.

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