14C-urea breath test for assessment of gastric Helicobacter pylori colonization and eradication

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Background and Objective: Urea breath test (UBT) is a reliable noninvasive technique for detecting gastric Helicobacter pylori colonization. 14C isotope-based test requires simple equipment and is inexpensive. We studied the utility of 14C-UBT in diagnosis of gastric H. pylori infection. Methods: Presence of H. pylori was studied using antral histology and culture in patients with rapid urease test (RUT)-positive peptic ulcer. 14C-UBT was performed using a 185-hBg dose. Radioactivity in 15-min breath samples was measured using a β-scintillation counter and result expressed as % dose recovered:mmol CO2. H. pylori was considered positive when any two tests were positive. All tests were repeated one month after completion of H. pylori eradication therapy. Results: Among 41 patients (duodenal ulcer 36, gastric ulcer 5), H. pylori was detected by histology in 23 (56%) and by culture in 27 (66%). Overall, H. pylori was detected in 28 (68%) patients. Follow-up assessment was possible in 28 patients: 26 cleared the infection (all three tests negative). Mean 14C recovery values at 15 minutes associated with H. pylori-positive status were significantly higher (12.3 [SD 6.8] x 10^-3; n=30; p<0.001) than those associated with H. pylori-negative status (2.1 [0.9] x 10^-3; n=26). Using receiver-operating-characteristic analysis of 15-minute 14C recovery values, a cut-off of 6.5x10^-3 gave the best separation of H. pylori-positive and -negative cases. 14C-UBT had 93% sensitivity, 96% specificity and 95% accuracy. Conclusion: 14C-UBT appears to be a reliable noninvasive test for diagnosis of H. pylori infection. [Indian J Gastroenterol 2001;20:140-143]

Key words: Endoscopy, peptic ulcer

A number of tests have been developed to diagnose Helicobacter pylori infection and to evaluate the success of eradication therapy. There is no universally-accepted single test that can be considered 'gold standard'. Hence, one has to rely on two or more positive tests. Most of the commonly used tests are invasive (endoscopic biopsy-based) and may miss patchy infection. Moreover, invasive tests are not preferred for follow-up studies for assessment of eradication and recrudescence, and for evaluation of asymptomatic subjects.1

Urea breath test (UBT) is a noninvasive technique in which urea labeled with isotopic carbon is hydrolyzed by urease present in the organism, and free isotopic carbon atom is exhaled as CO2. This test can detect even patchy colonization anywhere in the stomach and therefore is highly sensitive and specific.2

Non-availability of atomic mass spectrometer and high cost of 13C test have been the main limitations in its widespread use, particularly in developing countries. However, a 14C-labeled test requires simpler equipment like a beta counter, which is available in many centers. It is inexpensive and the dose of radioactivity is within acceptable limits. We evaluated the sensitivity and specificity of 14C-labeled UBT in determining gastric H. pylori colonization and eradication after treatment.

Methods

Patients aged 18-60 years and having endoscopically-proven duodenal or gastric ulcer, defined as mucosal break greater than 5 mm in size and with an apparent depth, and a positive rapid urease test (RUT) were studied. Exclusion criteria were: lactating or pregnant women, coexisting gastric carcinoma or pyloric stenosis, previous gastric surgery, active upper GI bleeding, chronic alcohol or drug abuse (likely to be noncompliant), smoking, significant co-morbid illness, continued use of NSAIDs, and treatment with antibiotics and proton pump inhibitor in the preceding four weeks.

Detailed clinical history and physical examination findings were recorded in a structured proforma. Six biopsy pieces were collected from antral mucosa (5 cm from pyloroduodenal opening). One piece was examined for RUT. 3 pieces for histology and 2 pieces were used for H. pylori culture. Patients with positive RUT were included in the study. They underwent 14C-urea breath test (14C UBT) and were given therapy for H. pylori eradication using twice-daily doses of lansoprazole 30 mg, amoxicillin 1 g, clarithromycin 500 mg, and colloidal bismuth 250 mg for ten days. H2-receptor antagonists were prescribed thereafter if the patient had dyspeptic symptoms. A month after completion of H. pylori eradication treatment, patients were called for follow-up endoscopy, determination of H. pylori eradication in antral biopsy by RUT, histology and culture, and 14C UBT. H. pylori was considered eradicated if all the...
tests to detect *H. pylori* were negative. The study protocol was approved by the Institute's Research Committee and an informed consent was taken from all patients.

**Diagnostic tests**

**Rapid urease test:** Test reagent was prepared by adding 2 g urea in 10 mL of 0.5% phenol red and 20 mg sodium azide to 100 mL of 0.1M sodium phosphate buffer. After 24 h, the pH was adjusted to 6.5. The antral biopsy was placed in 1 mL of the reagent placed earlier in the well of a microtiter tray; color change from yellow to pink within 1 hour was taken as a positive test.

**Antral histology:** Antral biopsy sections of approximately 3-μm thickness were examined after staining with HE and Giemsa stains.

**Culture for *H. pylori***: The antral biopsy tissue was transported and inoculated on fresh Columbia blood agar medium within 30 minutes. Culture was done in duplicate, with and without antibiotics (bacitracin, cycloheximide, colistin sulfate, cephalin and novobiocin). The plates were examined after 72 h of incubation under microaerophilic condition and then every 48 h for 7 days. Characteristic translucent and tiny colonies when found were subjected to smear examination, and tests for motility, oxidase, catalase, urease and hippurate hydrolysis.

**14C-urea breath test:** This was performed on the day of endoscopy, after thorough tooth-brushing. Each patient drank 185 kBq of 14C-urea (pharmaceutical grade; BARC, Mumbai) dissolved in 200 mL of water; this was followed immediately by rinsing of the mouth. Breath samples were collected at baseline and 15 minutes after the ingestion of radiouclide urea. Patient exhaled through a drinking straw with a side hole at its lower end, into a 20 mL scintillation vial containing 0.5 mmol of hyamine solution (benzothionium hydroxide) in 2 mL of absolute alcohol with thymolphthalein blue as the pH indicator till the solution changed color from blue to colorless signifying collection of 0.5 mmol CO₂. After adding 10 mL of toluene-based scintillation fluid, radioactivity was counted for 5 minutes in a liquid scintillation counter (Beckman LS1701 Analyzer; California, USA) together with a standard. The results were expressed as 14C recovery = % dose recovered/mmol CO₂. Reproducibility of 14C UBT was tested in 6 *H. pylori*-positive and 5 *H. pylori*-negative patients by repeating the test on the subsequent day.

**Definitions**

A patient was defined as 'H. pylori-positive' if RUT and either histology or culture were positive, and 'H. pylori-negative' when all three tests were negative. The patients were labeled as 'only RUT positive' when RUT was positive but culture and histology were negative.

**Statistical methods**

Quantitative variables were compared by Student's *t*-test. To assess the performance of the 14C UBT, 15-minute 14C recovery values for *H. pylori*-positive and *H. pylori*-negative patients were plotted graphically. Receiver-operator-characteristic (ROC) analysis was applied to the above data set to determine a cut-off value of 14C UBT that best distinguishes between *H. pylori*-positive and *H. pylori*-negative patients.

**Results**

The 41 study subjects (31 men) with RUT-positive peptic ulcer disease (duodenal ulcer 36, gastric ulcer 5) had a mean age of 36.3 (SD 10.1; range 18-58) years. Mean duodenal ulcer size was 8 mm (range 6-11) and gastric ulcer 14 mm (range 7-22); seven patients had multiple ulcers. Other endoscopic findings were antral gastritis (36), corpus gastritis (7), duodenal bulb deformity (15) and esophagitis (5). Histology and culture positivity was 56% (23) and 66% (27), respectively; 28 patients (68%) were labeled as 'H. pylori-positive' and the remaining 13 as 'only RUT positive'.

Twenty-eight patients completed the follow up; 3 patients had mild adverse events and stopped treatment on their own. 4 became asymptomatic and refused repeat endoscopy, and 6 were lost to follow up. Complete ulcer healing was noted in 27 of 28 patients. Eradication of *H. pylori* was observed in 26 patients. The remaining two patients were RUT- and histology-positive but culture-negative; one of them had a persistent ulcer.

**14C recovery values**

Of the total 69 UBT values available, 26 belonged to *H. pylori*-negative status, 30 to *H. pylori*-positive status, and 13 to only RUT positive status.

Fifteen-minute 14C recovery values were significantly higher in the *H. pylori*-positive group (mean [SD]
12.3 [6.8] x 10⁻²; median 11.8 x 10⁻³, range 4.8 to 53.6 x 10⁻³) compared to the H. pylori-negative group (2.1 [0.9] x 10⁻³, 2.2 x 10⁻³, 0.7 to 9.2 x 10⁻³). Mean ¹⁴C UBT values in the only RUT-positive group (11.8 [5.3] x 10⁻³; 10.6 x 10⁻³, 3.9 to 41.3 x 10⁻³) was similar to the H. pylori-positive group and higher than in the H. pylori-negative group (p<0.001).

On ROC analysis a cut-off of 6.5 x 10⁻³ for 15-minute ¹⁴C recovery value gave the best separation between H. pylori-positive and -negative groups (Fig), with sensitivity of 93%, specificity of 96%, positive predictive rate of 97%, negative predictive rate of 93%, and overall accuracy of 95%. When this cut-off was applied to the group with only RUT positivity, 12 of 13 (92%) had UBT values in the H. pylori-positive range.

In 10 of 11 patients (6 H. pylori-positive and 5 H. pylori-negative) who were studied twice within two days, variation in 15-minute ¹⁴C recovery values was less than 5%; in the remaining patient, the variation was 7%. No patient required reclassification after the second test.

**Discussion**

Our study validates the clinical usefulness of ¹⁴C UBT by showing that it has a high sensitivity (93%) and specificity (96%) and a diagnostic yield superior to that with histology (56%) and culture (66%). Using a 5-mCi (185 kBq) dose of ¹⁴C urea, similar sensitivity (91%-100%) and specificity (89%-100%) rates have been reported previously.²,³

The diagnostic cut-off level suggested by Raju et al.² in a 20-minute breath sample was 6.6 x 10⁻³ and by Kaul et al.⁵ was 7 x 10⁻³ in a 15-minute breath sample. These do not differ from the cut-off level obtained in the present study (6.5 x 10⁻³ in 15-minute breath sample). RUT positivity alone is usually not considered unequivocal evidence of H. pylori positivity. In our study, the ¹⁴C UBT was almost invariably (92%) positive in the "only RUT positive" group. This suggests that even RUT positivity alone may be an adequate indication of H. pylori positivity when other tests give negative results.

Apart from simplicity and high accuracy, ¹⁴C UBT has the advantage of long half-life (5.7 x 10³ years) of the ¹⁴C isotope. Hence, the isotope and breath sample can be stored for a long period. Five mCi dose of ¹⁴C was used in the current and previous studies to keep radiation within acceptable limit. The dose of ¹⁴C isotope can be brought down to 1 mCi by using a larger quantity of breath sample.⁸ We used the 5 mCi dose because of problems of availability and cost of breath sample.

We studied only a single 15-minute breath sample. The efficacy of a single 15-minute sample has already been established in several studies.⁵,⁷,⁸ This time interval was chosen to avoid the theoretical risk of false positivity in earlier breath samples due to urease activity of buccal flora. In addition, it enables the detection of patients with low bacterial load where the peak of ¹⁴CO₂ generation may be delayed.⁴ We expressed the breath test results as percent ¹⁴CO₂/mmol CO₂ of the administered dose rather than in terms of counts per minute or disintegration per minute to make the result independent of the amount of CO₂ collected and the dose of isotope administered.

¹⁴C isotope has not been approved for clinical application in Western countries because of the radiation risk involved. It however has the advantages of being inexpensive, stable and being detectable with simpler equipment. Further, the radiation risk associated with ¹⁴C has been found to be within acceptable limits; 5 mCi of ¹⁴C provides a whole-body radiation dose of 20-50 mSv, which is comparable to the radiation exposure from a chest X-ray, nearly a thousand times less than that from a barium meal examination, and comparable to background radiation.⁴ Concerns about the long half-life of ¹⁴C are also not valid since biological half-life of the isotope in the body is short. About 75% of the ¹⁴C is exhaled in the breath within 5 h of oral administration, 22% is used in metabolic processes with an excretion half-life of 10-12 days, and 3% enters the bone with an excretion half-life of 40 days.⁶,⁹,¹⁰ So far there is no experimental evidence for a possibility of gene mutations.¹¹

In conclusion, ¹⁴C urea breath test has high specificity, sensitivity and overall accuracy for the diagnosis of H. pylori infection, leads to radiation exposure within relatively safe limits, and has a diagnostic yield better than that of histology and culture. It may be of particular value in clinical situations in which endoscopy is not indicated, such as assessment of post-treatment gastric H. pylori status and in epidemiologic studies.

**References**

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**NEWS AND NOTICES**

**A Workshop and Continuing Surgical Education Program on Benign Anorectal Anomalies will be held in Pondicherry July 21 and 22, 2001.** For details, contact: Dr K P Singh, Convenor, 33 HIG House, Ashok Nagar, Pondicherry 605 008 Tel: (413) 338105 (O); 251966, 250667 (R); 98430 31966.

The 14th World Conference of the International Society for Laser Surgery and Medicine will be held in Chennai August 27 - 30, 2001. For details, contact: Dr B Krishna Rao, President, No. 5 Chandra Bagh Avenue, Second Street, Mysapore, Chennai 600 004 Tel: (44) 859 4804, 852 7776, 476 5856 Fax: (44) 859 4576, 476 7008 E-mail: bkr@vsnl.com Website: http://www.medindia.net/lisl2001

The 11th Annual Conference of Pediatric Gastroenterology will be held in Chandigarh August 31 - September 2, 2001. For details, contact: Dr B R Thapa, Organizing Secretary, Ped Gastro Con 2001, Division of Pediatric Gastroenterology, Hepatology and Nutrition, Superspeciality of Gastroenterology, PGIMER, Chandigarh 160 012 Tel: (172) 71 5352 (R); 74 7585-600, 74 7610-25, Extn 465 (O) E-mail: medinst@pgi.chd.nic.in; bthapa1@yahoo.co.in Website: http://www.pgimer.nic.in

The 9th Asian Conference on Diarrheal Diseases and Nutrition will be held in New Delhi September 28 - 30, 2001. For details, contact: Prof M K Bhan, Conference Secretary, ASCODD2001, Room No. 3054, Academic Block, Department of Pediatrics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110 029 Tel: (11) 696 3822, 659 4792, 656 1123, 656 0110 Extn 3290. Fax: (11) 686 2663 E-mail: asccd2001@delhi.is Website: http://www.ascodd2001.deli.as

The 42nd Annual Conference of the Indian Society of Gastroenterology and associated societies will be held in Varanasi from March 2002. For details, contact: Prof S R Naik, Department of Gastroenterology, SGPGI, Lucknow 226 014 Tel: (522) 44 0700, 44 0800 Extn 2400 Fax: (522) 44 0078, 44 0017 Website: http://www.sgpgi.ac.in/conf/issg2001.html

The VI International Surgical Conference of the Society of Surgeons of Nepal will be held in Kathmandu, November 21 - 23, 2002. For details, contact: Dr Manohar Lal Shrestha, Organizing Secretary, Society of Surgeons of Nepal, NMA Building, Exhibition Road, Kathmandu, Nepal Fax: 977 (1) 22 5300. E-mail: ssn@healthnet.org.np