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DERLEME/REVIEW ARTICLE

Diagnostic methods of *Helicobacter pylori* infection

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Summary
Helicobacter pylori is a spiral, gram negative bacterium and found to be associated with gastritis, peptic ulcer disease, gastric adenocarcinoma and MALT lymphoma. Thus there is an increasing importance of the treatment of *Helicobacter pylori* and thereby a great need for simple and accurate diagnostic methods. *Helicobacter pylori* infection can be diagnosed by invasive and non-invasive techniques. Invasive diagnostic methods require mucosal biopsy during endoscopy and then subjecting them to culture, rapid urease test, polymerase chain reaction or histologic analysis. Non-invasive methods include antibody detection (serology), stool antigen and urea breath tests.
Key words: *Helicobacter pylori*, rapid urease test, urea breath test

Özet
***Helicobacter pylori* enfeksiyonu tanısında tanısal yöntemler**
Helicobacter pylori, spiral yapısında gram negatif bir bakteridir ve gastrit, peptic ülser, gastrik adenokarsinoma ve MALT lenfoma ile ilişkili olduğu gösterilmiştir. Bu nedenle tedavisi gittikçe önem kazanmaktadır ve basit, doğru tanısal yöntemlere gereksinim vardır. *Helicobacter pylori* enfeksiyonu tanısı invaziv ve noninvaziv yöntemler ile konulabilir. İnvaziv tanısal yöntemler endoskopik işlem sırasında biyopsi alınmasını ve bu doku parçalarının daha sonra kültür, hızlı üreaz testi, polimeraz zincir reaksiyonu veya histolojik analize tabi tutulmasını gerektirir. Noninvaziv yöntemler ise, serumda antikor saptanması (seroloji), gaitada antijen ve üre nefes testlerini içerir.
Anahtar kelimeler: *Helicobacter pylori*, hızlı üreaz testi, üre nefes testi

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Introduction

Helicobacter pylori (*H.pylori*) is a spiral-shaped, gram-negative bacterium that is found in the gastric mucous layer or adherent to the epithelial lining of the stomach. Most persons who are infected by *H.pylori* never suffer any symptoms related to the infection; however *H.pylori* causes chronic gastritis (95%), gastroduodenal ulcers (70-80%) and duodenal structural and functional abnormalities. Infected persons have 2- to 6-fold increased risk of developing gastric cancer and mucosal-associated-lymphoid-type (MALT) lymphoma (1). It has been classified as a Class I carcinogen. The majority of patients having symptoms or signs of these diseases were given long term medications such as H_2 blockers and proton pump inhibitors. These medications relieve ulcer-related symptoms, heal gastric mucosal inflammation, and may heal the ulcer but they do not treat the infection and there is no chance of permanent cure and the majority of ulcers recur. Eradication of this infection with appropriate antibiotic therapy has been shown to decrease ulcer recurrence $<10\%$ in one year versus a 60-100% one year recurrence with use of anti-ulcer medications alone. Given the clinical importance of *H.pylori,* there is a need for prompt and accurate diagnosis followed by eradication therapy and confirmation of eradication after therapy.

H.pylori infection can be diagnosed by invasive and non-invasive techniques (Table I) (2). Invasive techniques require endoscopy and mucosal biopsy and specimens that will later be subjected to culture, rapid urease test or histological examination. However endoscopy is expensive, unpleasant for patients and carries a small but definite risk of complications. Thus, the

use of non-invasive tests to diagnose *H.pylori* infection is becoming more frequent. Indeed, recent studies have demonstrated that a strategy of 'test and treat' for *H.pylori* in uninvestigated, young (<50 years), dyspeptic patients in primary care is safe and reduces the need for endoscopy. Non-invasive methods include serology, stool antigen test and urea breath tests (UBT). There are two different types of non-invasive tests: direct and indirect (Table I). The direct tests look for evidence of the presence of *H.pylori.* The stool test assesses the presence of bacterial antigens in stool. Indirect tests assess the presence of the infection by evaluating indirect evidence such as the presence of antibodies to *H.pylori,* or the amount of labeled CO_2 in the breath, an expression of the urease activity of the bacterium.

Table I. Diagnostic tests for *Helicobacter pylori* infection

Invasive techniques	Noninvasive techniques	
	Direct	Indirect
Histology Rapid urease test Culture	Stool antigen test	Urea breath test Serological tests

Invasive techniques

Histology

Histological examination of biopsy samples taken during endoscopy is usually considered 'the gold standard' for the diagnosis of *H.pylori.* But owing to the patchy distribution of *H.pylori* in gastric mucosa, the biopsy-based tests may suffer from sampling error (3,4). Furthermore, histological examination is highly dependent on the experience of the pathologist, and high inter-observer variation has been reported (4,5).

Rapid urease test (CLO test)

Biopsies of gastric mucosa are placed in a gel containing urea, and the subsequent ammonia production causes a pH change, which is observed as a color change. Besides suffering from biopsy sampling error, the CLO test depends greatly on the pH of the media and the amount of the urea in the medium. These factors may vary in different products and thereby influence the results obtained with other tests (3,6).

Culture

Culture is the most specific diagnostic method for *H.pylori* infection but its sensitivity is low. The role of culture for primary diagnosis is limited but it is an important method as isolates for the traditional susceptibility testing are obtained. Although routine susceptibility testing for *H.pylori* is not recommended, increasing resistance rates to metronidazole and clarithromycin might make routine susceptibility more popular (7).

Non-invasive techniques

Serological tests

Serological tests are based on the detection of specific anti-*H.pylori* IgG antibodies in a patient's serum. While serological tests are simple and easy to perform, they are not reliable tests for the diagnosis of *H.pylori* infection in elderly people because of poor antibody production, or for determination of eradication of *H.pylori,* since it remains positive for a long period despite adequate treatment (6,8). Serological tests are not able to distinguish between active infection and a previous exposure to *H.pylori.* Different commercial kits also have different levels of diagnostic accuracy (range 68-82%) (9,10).

Stool antigen test

An enzyme immunoassay, which detects the presence of antigen in stool specimen, has recently become available. This assay has undergone extensive testing for the initial diagnosis of the *H.pylori* infection and in the confirmation of eradication after treatment. Several studies have suggested that polyclonal antibody test is comparable to the ure breath test in the initial diagnosis of *H.pylori* infection (sensitivity 93.2% and specificity 93.2%). It has been reported that stool antigen test is less accurate than UBT in the post-treatment setting (11). Recently it has been reported that monoclonal technique has higher sensitivity than the polyclonal one especially in the post-treatment setting (12).

Urea breath tests

H.pylori produces urease, an enzyme that splits urea into ammonia and carbon dioxide. The production of high amounts of urease by *H.pylori* has been used in the development of urea breath tests (Figure 1). Patients ingest urea labeled ^{13}C or ^{14}C. Hydrolysis of urea occur within the mucous layer and results in the production of ammonia and labeled CO_2. Labeled CO_2 diffuses into the blood vessel and can be detected in the breath as a marker of infection (Figure 1). UBTs with either ^{13}C or ^{14}C-urea sample the whole stomach and reflect the active infection. Both tests have been proved to be very accurate, with reported sensitivities of 97-100% and specificities of 95-100% for both diagnosis and proof of eradication of *H.pylori* infection after therapy (5,13-18), and it is even used as gold standard in some studies. While the two isotopes seem to offer similar diagnostic accuracy, ^{13}C-UBT has the inconvenience of requiring (a) more complex and expensive equipment on site or else analysis off-site by an external laboratory and (b) administration of a test meal and cold urea to the patient. These are not necessary with ^{14}C, and the test is thus more simple, faster and cheaper. The diag-

nostic reproducibility of the ^{14}C-UBT is very good (19).

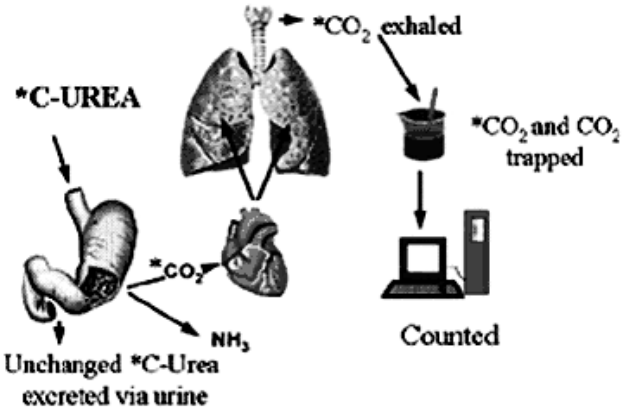

Figure 1. The principle of urea breath test

The routine test protocol of ^{14}C-UBT requires ingestion of ^{14}C-urea, collection of breath samples at frequent intervals using a liquid CO_2 trapping medium (usually hyamine), addition of a liquid scintillation cocktail and counting with a ß-scintillation counter. Several different methodological approaches have been proposed to develop a more simple and quicker test. Recently a new device for ^{14}C-UBT has been described (20). It uses a flat, dry breath card, which is able to adsorb exhaled via chemical binding (Heliprobe BreathCard). This card is then read using a small analyser with a slot into which the card is put (Heliprobe analyzer). In addition to the simple and easy collection of breath, this system prevents accidental ingestion of hazardous organic CO_2 absorber solutions during breath sampling. Clinical validation of this new system showed high diagnostic accuracy for both diagnosis and proof of eradication of *H.pylori* infection after therapy (20,21). Additional advantages of the Heliprobe system are the shorter test time and the low cost. Breath samples are analyzed with a ß-scintillation counter in ^{14}C-UBT and with a mass spectrometer in ^{13}C-UBT. Because both items of equipment are expensive, analysis can be done in an external laboratory by mail order and results are usually obtained a few days later. In contrast, with the Heliprobe system the results are obtained in half an hour on-site and the analyzer is much cheaper than either a ß-scintillation counter or a mass spectrometer.

Various factors affect the results of the UBT and this test has been extensively modified since its first description. The differences concern variations in the doses and forms of ^{14}C-urea, patient preparation before the test, sampling time, number of breath samples and modes of quantification.

The original ^{14}C-UBT system used relatively high activities (200-400 kBq) and multiple breath sampling. Later studies showed that the diagnostic accuracy of ^{14}C UBT is maintained even with low doses such as 37 kBq (1 μCi) and single breath sample (15,22).

The UBT indirectly detects gastric *H.pylori* by measuring urease activity. Sources of urease other than *H.pylori,* such as bacterial overgrowth in the stomach or upper intestine, may rarely cause false-positive test results (22). Urease-producing bacteria are also present in the oropharynx and may cause false-positive results, especially in early breath samples. Late breath sampling may result in false-negative results because of emptying of urea from the stomach. Several procedures to avoid contamination of breath by the oropharyngeal flora have been suggested, including mouth washing, simultaneous meal to delay gastric emptying, and performance of multiple breath sampling. Another more simple and effective method is the use of ^{14}C-urea in a gelatin capsule, thus bypassing the oropharynx. Hamlet et al. have reported that when the ^{14}C-urea is supplied in a capsule, a single 10-min breath sample is highly accurate (100% sensitivity and specificity) for the diagnosis of *H.pylori* infection. They have compared the capsule method with the urea drink method and found the former to be more reliable because no overlapping in activity has occurred between *H.pylori* -positive and -negative patients; by contrast, conventional breath testing has showed overlapping during the whole 30-min test period. Their study has also showed that a fatty test meal lowers the $^{14}CO_2$ excretion during the first 20 min and may adversely affect the accuracy of a rapid UBT (16). Other advantages of the capsule form include commercial availability, no risk of spills, shorter test duration and a lower radiation dose.

The expression of results of UBT varies between investigators. Henze et al. and Veldhuyzen van Zanten et al. have used CPM (23,24). Because CPM is affected by chemical or color quenching, chemical changes of the cocktail and methods of sample preparation, Pathak et al. have strongly suggested the use of DPM counts (25).

Some authors have used formulas to correct for body weight or body surface to account for differences in endogenous CO_2 production, the results being expressed as recovery standard units [(% of administered dose recovered/mmol CO_2 trapped) × body weight (kg)] (13,15). However, neither of these factors has been proved to influence the results of the breath test. Indeed, it has even been reported that uncorrected

counts result in better distinction between *H.pylori* -positive and -negative patients (16,24,25).

Adequate patient preparation is important if accurate results are to be obtained with ^{14}C-UBT. Fasting state of patients is important first step for the test (26). A large number of investigators have reported that the UBT becomes false negative during therapy with proton pump inhibitors, lansoprazole, bismuth compounds, antibiotics and ranitidine (27,28). Preliminary reports indicate that addition of citric acid to the urea solution/capsule may diminish the negative effect of acid-inhibitory drugs on the accuracy of UBT (30). The exact value of acidified urea needs further verification.

Carbon-13 is a non-radioactive isotope, but ^{13}C-UBT is more expensive because it requires mass spectrometry. ^{14}C has a physical half-life of about 5,000 years, raising the question of the risks of radiation exposure. Because nearly the entire ingested isotope is rapidly excreted in urine or breath over the following 72 h and only a small amount of isotope is used, the test actually entails low radiation exposure (3 μSv) (31,32). In fact, the dose is less than the natural background radiation in one day; it is similar to the additional radiation received from cosmic rays during a 1-h jet flight. In terms of other radiological investigations, the dose from one ^{14}C-UBT is equivalent to roughly one-seventh of that from a chest x-ray (20 μSv), or one-thousandth of that from a barium meal (3 mSv). For this reason, in 1997 the Nuclear Regulatory Commission permitted in vivo diagnostic use of capsules containing 1 μCi of ^{14}C-urea without a license (33). This margin of safety makes it acceptable to use the test for sequential studies.

Conclusions

* Non-invasive tests are becoming more important in the clinical management of dyspeptic patients.
* A number of clinical guidelines recommend non-invasive testing in dyspeptic patients, followed by treatment of *H.pylori.*
* European *Helicobacter pylori* study group has recommended the use of urea breath test or stool testing in initial diagnosis of *H.pylori* infection and stool test may be an alternative to UBT after treatment.
* Different serological tests have varying levels of accuracy and are not appropriate for confirming eradication.
* The accuracy of low dose, single sample ^{14}C-UBT is high.
* A low dose capsule ^{14}C-UBT gives less than the natural background radiation in one day.

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