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Evaluation of the "One-minute" test for detecting *Helicobacter (Campylobacter) pylori* infection

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Summary

The "one-minute" urease test to detect *Helicobacter (Campylobacter) pylori* infection was evaluated using histology and culture as the 'gold standard". The test was performed in a blinded manner and compared with the conventional Christensen's urease test. *Helicobacter pylori* was detected in 88 of 100 consecutive patients attending the gastrointestinal clinic for upper endoscopy. Although the "one-minute" urease test was more sensitive [86% (76/88)] than the conventional Christensen's urease test [70% (62/88)], this difference was not statistically significant (P = 0.22). Histology was the most sensitive [97% (85/88)] whilst culture was 80% (70/88) sensitive. All tests exhibited specifications of 100%. The "one-minute" urease test is a simple, rapid and highly specific test to detect *Helicobacter pylori* which can be performed at endoscopy.

Résumé

En employant l'histologie et la culture comme "standard d'or", on a évalué le test d'uréase "d'une minute" pour détecter l'infection d'*Helicobacter (Campylobacter) pylore*. Le test a été effectué d'une manière blindée et par la suite il a été comparé au test conventionnel d'uréase de Christensen. On a détecté l'*Helicobacter pylore* chez 88 sur 100 malades consécutifs qui assistaient à la Clinique Gastro-intestinale pour la haute endoscopie. Bien que le test d'uréase "d'une minute" ait montré plus de sensibilité (86%, c'est-à- dire 76/88) que le test conventionnel de Christensen (70%, c-à-d 62/88) cette différence n'est pas significative sur le plan statistique. L'histologie a indiqué la plus grande sensibilité (97%, c-à-d 85/88), alors que la culture en a fait preuve à 80% (70/88). Tous les tests ont exhibé des spécificités de 100%. Le test d'uréase "d'une minute" est facile, rapide et très spécifique pour détecter l'*Helicobacter pylore*; it peut être effectué à titre d'endoscopie.

Introduction

Campylobacter pylori now renamed *Helicobacter pylori* is strongly associated with chronic active gastritis[1-3]. *Helicobacter pylori* can be demonstrated in gastric antral biopsies by culture and/or histology[2,3]. Both of these tests are time-consuming and take several days to report. Thus a rapid test that can specifically identify *H.pylori* infected patients, could expedite decisions regarding treatment. Rapid hydrolysis of urea by *H. pylori* preformed urease is unique when compared with the ureases produced by other bacterial species, *viz.* Proteus, Providencia, Monganella and Klebsiella[4-6]. Several investigators have used this characteristic to simplify the diagnosis of *H. pylori* infection[7]. We report our local experience with the "one- minute" urease test used by Arvind *et al* [8] for the detection of *H. pylori* in gastric biopsy specimens.

Materials and methods

One hundred newly presenting patients, in whom upper gastrointestinal endoscopy was performed for symptoms referable to the upper gastrointestinal tract, were studied. From each patient, three antral mucosal biopsies within 5cms of the pylorus were obtained for histology, culture and urease assay.

A specimen for histology was fixed in 10% formalin and routinely processed. Sections were stained with haematoxylin and eosin (H & E) and examined to determine the presence of gastritis and *H. pylori* [2,3]. Any gastritis present was classified into chronic superficial or chronic atrophic gastritis

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and the pattern of inflammation was further categorised as "active" or "inactive". "Active" gastritis required the presence of polymorphs and also lymphocytes and plasma cells infiltrating the lamina propria whereas polymorphs were absent in those classified as "inactive". A presumptive diagnosis of *H. pylori* was made on seeing curved or spiral organisms, usually lying in the mucous layer on the mucosal surface and within the superficial glands.

A second antral biopsy was transported to the microbiology laboratory and processed within 2 hours of collection. This specimen was first inoculated by rubbing it over the surface of a chocolate agar plate with sterile forceps and was then placed in 2ml of 2% Christensen's urea broth. The inoculated chocolate agar plate was incubated microaerophilically at 37°C[2,3]. Plates were examined on days 3, 7 and 10 for bacterial growth.

Suspect colonies were identified as *H. pylori* by characteristic colonial morphology, positive oxidase test, positive catalase test and a characteristic urease reaction. A colour change from yellow to pink or orange indicated a positive test. The specimen was considered urease-negative if no colour change developed after 18 hours incubation.

A third biopsy was placed immediately into the urea-phenol red solution in the endoscopy room, and kept at room temperature[8]. A positive result was recorded as soon as a colour change from yellow to pink was observed around the biopsy specimen. Both urease assays were performed independent and in a blinded fashion, with no knowledge of histology and culture results.

Assay performance

Because of the fastidious nature of *H. pylori*, culture is problematic[2,3]. Morever, sampling error due to the patchy distribution of *H. pylori* in the stomach, use of antibiotics and the technical difficulties associated with culture reduces the diagnostic yield from patients[2,3]. Most investigators agree that histology or a combination of histology and culture represents the "gold standard" for diagnosis[9]. Specificity was monitored by evaluating the tests in histologically and bacteriologically well defined groups of patients. Almost 100% of our patients with gastritis and duodenal ulcers are infected with *H. pylori*[2,3]. Because of the high prevalence of *H. pylori* infection in our African population we predict that it will be difficult to find enough *H. pylori* negative controls (histology and culture negative) to monitor specificity, hence the uncontrolled bias of the small number of patients in our control group (n=12).

Histology and/or culture was used as the "gold standard" to define the sensitivity and specificity of the tests[9]. Sensitivity was expressed as the percentage of the positive test results in patients with *H. pylori* infection who were positive by histology and/or culture or both. Specificity was expressed as the percentage of negative test results in patients without *H. pylori* infection who were negative by histology and culture. The method of Galen (1986) was used to calculate sensitivity and specificity according to the formulae:

Sensitivity = a/a + c x 100%

Specificity = d/b + d x 100%

Results

H. pylori was detected in 88 of 100 patients by either culture and/or histology or both. Detection of *H. pylori* by culture and/or histology, or both, was used as the "gold standard" with which the urease assays were compared. Comparative results are shown in Table 1. Histology was the most sensitive (97%) and specific (100%) technique evaluated. Although culture was specific (100%) it was less sensitive (80%) than histology. The "one-minute" urease test detected 86% of *H. pylori* positive patients and was 100% specific, in contrast to the Christensen's urease test, which detected 70% of *H. pylori* positive patients. Using Fisher's Exact test, there was no significant difference (P = 0.22) between the urease assays.

Test	Disease present	Disease absent	Total
positive	a	b	a + b
negative	c	d	c + d
Totals	a + c	b + d	a+b+c+d=N

Table 1: Comparison of the "One-minute" urease test with histology, culture, and Christensen's Urease test (37°C)

Method	*H. pylori* Positive* (88)		*H. pylori* negative** (12)		Sensitivity	Specificity
	No. Positive	No. Negative	No. Positive	No. Negative		
Histology	85	3	0	12	97%	100%
Culture	70	18	0	12	80%	100%
Christensen's urease test (37°C)	62	26	0	12	70%	100%
"One-minute" urease test	76	12	0	12	86%	100%

* *H. pylori* positive = organism either cultured and/or seen on histology
** *H. pylori* negative = organism neither cultured nor seen on histology

Discussion

The aim of our study was to determine which of the existing test with a high specificity was rapid enough so that patients with *H. pylori* infections could be accurately identified without having to wait up to a week for the results of culture and histology. The sensitivity (86%) and specificity (100%) of the "one-minute" urease test is similar to that reported by others[8,11,12]. There were no false positive results with the "one-minute" urease test which is an advantage, since the shorter incubation time will exclude interference from other urease-producing contaminants[8]. The 100% specificity of the urease test is ideal for rapid identification of patients with *H. pylori* infection. However, the lower sensitivity (86%) would suggest that some infected patients will be missed on initial screening. These patients would be identified by histology and culture results[11]. Sensitivity of the test could be improved by increasing the observation time. Ruiz *et al.* [12] has suggested extending the observation period to 30 minutes.

The predictive values of the tests evaluated in this study are of limited value because of the "uncontrolled bias" of our data collection. Ninety-seven per cent (97%) of our patients have *H. pylori* infection, an observation we reported previously[2]. There have been several reports on urease assays for the rapid detection of *H. pylori* [7]. Most of these tests appear to be specific (100%), but their sensitivities vary from 60% to 100% depending on the media, incubation times and temperatures used[7,13,14].

The results of this study confirms our previous observations that in our hands, histology is the most sensitive (97%) test for the detection of *H. pylori* in comparison to culture and Christensen's urease test[2,3]. All of these tests are useful in detecting *H. pylori*, but the "one-minute" urease test can be performed with greater ease and less expense[15].

In view of the strong association of *H. pylori* with gastritis and duodenal ulceration[2,3], and the potential role of antibiotics in the treatment or prevention of these conditions, rapid tests such as the "one-minute" urease test may prove to be an important tool in initial patient assessment and follow-up[16]. If the test is positive, appropriate therapy may be initiated immediately. The test could be useful for the prompt enrolment of patients into therapeutic trials.

The "one-minute" urease test is a rapid, simple test that can be performed by an endoscopist with little or no microbiological experience.

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