Comparison of salivary and serum enzyme immunoassays for the diagnosis of *Helicobacter pylori* infection

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Infection with *Helicobacter pylori* has been established as an important risk factor for the development of peptic ulcer disease, gastritis and gastric cancer. The diagnosis of *H pylori* infection can be established by invasive or noninvasive techniques. Two noninvasive enzyme immunoassays (EIAs) for antibody detection – HeliSal and Pylori Stat – were compared with histology. Both assays detect immunoglobulin (Ig) G directed against purified *H pylori* antigen. The test populations consisted of 104 consecutive patients scheduled for upper gastrointestinal endoscopy. Of these patients, 97 (93%) had symptoms compatible with peptic ulcer disease. Saliva and serum were collected simultaneously at the time of endoscopy. Salivary EIA had a sensitivity of 66%, specificity of 67%, positive predictive value of 67% and negative predictive value of 66% compared with the serum EIA, where the results were 98%, 48%, 64% and 96%, respectively. Although the salivary EIA is an appealing noninvasive test, it was not a sensitive and specific assay. The serum EIA also lacked specificity, but was highly sensitive with a good negative predictive value. Although a negative serum EIA rules out *H pylori* infection, a positive result must be interpreted in the clinical context and confirmed with a more specific measure.

Key Words: *Enzyme immunoassay,* Helicobacter pylori, *Helisal, Pylori Stat, Saliva, Serum*

Comparaison des immunodosages enzymatiques salivaires et sériques pour le diagnostic de l'infection à *Helicobacter pylori*

RÉSUMÉ : L'infection à *Helicobacter pylori* a été confirmée en tant que facteur de risque important d'interaction de la maladie ulcéreuse gastro-duodénale, de la gastrite et du cancer de l'estomac. Le diagnostic de l'infection à *H. pylori* peut être confirmé au moyen de techniques vulnérantes ou non vulnérantes. Deux immunodosages enzymatiques non vulnérants pour le dépistage des anticorps, soit HeliSal et Pylori Stat, ont été comparés au moyen d'analyses histologiques. Les deux trousses permettent de déceler la présence de l'immunoglobuline G (IgG) dirigée contre l'antigène *H. pylori* purifié. Les populations testées regroupaient 104 patients consécutifs qui devaient subir une endoscopie des voies digestives supérieures. Parmi ces patients, 97 (93 %) présentaient des symptômes qui concordaient avec une maladie ulcéreuse gastro-duodénale. Des échantillons de salive et de sérum ont été recueillis simultanément au

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moment de l'endoscopie. Le dosage salivaire présentait une sensibilité de 66 %, une spécificité de 67 %, une valeur prédictive positive de 67 % et une valeur prédictive négative de 66 % en comparaison avec le dosage sérique pour lequel les résultats étaient de 98 %, 48 %, 64 % et 96 % respectivement. Bien que le dosage salivaire soit un test non vulnérant attrayant, il ne s'agit pas d'un test sensible et spécifique. Le dosage sérique a également manqué de spécificité, mais s'est révélé très sensible et doté d'une bonne valeur prédictive négative. Bien que l'immunodosage sérique négatif permette d'écarter le diagnostic d'infection à *H. pylori*, un résultat positif doit être interprété dans le contexte clinique et confirmé au moyen de mesures plus spécifiques.

Helicobacter pylori is a motile, microaerophilic, curved Gram-negative rod which has been established as an important risk factor for the development of peptic ulcer disease (PUD) and gastritis (1). The National Institute of Health, Bethesada, Maryland have recommended that all patients with PUD infected with *H pylori* should receive antimicrobial therapy because cure of this infection leads to markedly reduced ulcer recurrence rates (1).

The diagnosis of *H pylori* infection can be made either invasively by means of endoscopy and histological analysis of the biopsied tissue or noninvasively by the urea breath test and antibody detection in serum. Enzyme immunoassays (EIAs) that detect immunoglobulin (Ig) G in saliva have been developed as an alternative to the aforementioned techniques (2). Anti-*H pylori* IgG is found in saliva, and the advantage of the currently available salivary assay for *H pylori* is its ease of administration. If the salivary EIA is to be an effective noninvasive screening tool for *H pylori*-induced disease, it must be sensitive (2-6), specific and compare favourably with other already established noninvasive and invasive techniques, such as the urea breath test and serology (7-9). The objectives of this study were to determine the sensitivity, specificity, positive (PPV) and negative predictive values (NPV), and test accuracy of the Helisal (Axcan Pharma) Salivary EIA for diagnosing *H pylori* in comparison with the 'gold standard' of endoscopic biopsy for histology, as well as with a standard serum EIA (Pylori Stat, Bio-Whittaker, Maryland).

PATIENTS AND METHODS

Study design: One hundred and four sequential patients who were being evaluated by flexible upper gastrointestinal (GI) endoscopy by the gastroenterologist investigators between July 1, 1994 and December 31, 1994, were enrolled in the study after providing informed consent. Just before endoscopy, all subjects had blood drawn and saliva collected for serology. Two prepyloric greater curvature biopsies were obtained. Demographic details (age, sex, smoking history, antimicrobial and other medication use, living conditions) and clinical factors (indications for and findings at endoscopy) were obtained using a standardized questionnaire.

Histology: Histology was used as the gold standard against which different EIAs were compared (10). All biopsies were evaluated by an experienced GI pathologist who was unaware of the patient's clinical or other laboratory data. Biopsies were routinely stained with hematoxylin and eosin, and deemed positive, if the organism was visualized. If the interpretation was equivocal or there were signs of gastritis, further evaluation with Giemsa or Warthin-Starry silver stains was performed.

Salivary EIA: Saliva specimens were collected using the Omni-Sal saliva collection device (MML Diagnostic Packaging Inc, Oregon). The samples were analyzed by the HeliSal Salivary EIA in accordance with the manufacturer's instructions. The samples were read using a Behring ELISA Processor II (Behring Werke AG Diagnostics, Marberg, Germany) at 450 nm, using more than 0.3 enzyme units/mL as the cut-off for a positive test result. The investigator performing the salivary and serum EIAs was unaware of the patient's clinical status.

Serology: Whole blood (10 mL) was collected in glass serum tubes, and sera separated and stored at –20°C until assayed. Analysis was by means of the Pylori Stat Serum EIA according to the manufacturer's instructions. The samples were read using a Ceres UV 900 HDL spectrophotometer (Bio Tek Instruments Inc, Vermont) at 550 nm, using 1.0 predictive index values as the cut-off for a positive result.

Statistical analysis: The Statistical Package and Service Solution for Windows statistical package version 6.1 (SPSS Inc, Illinois) was used to analyze the data. The sensitivity, specificity, PPV, NPV and test accuracy for each assay was determined by constructing 2×2 contingency tables and using histopathology as the gold standard. χ^2 was used to determine the significance of discrete variables, while Student's *t* test was used to determine the significance of continuous variables.

RESULTS

Of the 104 patients, salivary EIA was available in 101. Of the 50 (49.5%) who were positive by histology, 33 were positive by salivary EIA and 17 were negative. Of the 51 (50.5%) who were negative by histology, 34 were also negative by salivary EIA. Serum EIA results were available for 100 patients. Of these, 48 were positive by histology and 47 by serum EIA. Of the 52 patients who were negative by histology, 25 were also negative by serum EIA and 27 were positive. The sensitivity, specificity, PPV, NPV and test accuracy of the salivary EIA were found to be 66.0%, 66.6%, 66.0%, 66.6% and 66.3%, respectively, and those for the serum EIA were 97.9%, 48.1%, 63.5%, 96.1% and 72.0%, respectively (Table 1).

For the group who was *H pylori* positive, the mean age was 50±19.3 years, 28 (56%) were male and 16 (32%) were smokers. Thirty-five (70%) lived in urban centres, and there were 3.0±1.7 inhabitants per dwelling. For the group who was *H pylori* negative, the mean age was 52.6±17.9 years, 24 (44%) were male and 12 (22%) were smokers. Forty-one (76%) lived in urban centres, and there were 3.16±4.09 inhabitants per dwelling. There was no statistically significant difference for these characteristics between the two groups. Table 2 describes medication use in the study population. There were no statistically significant differences between patients with and

TABLE 1
Comparison of published test characteristics for the detection of *Helicobacter pylori* with the result of the current study

Technique	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Test accuracy	Reference
Helisal (Axcan Pharma) (salivary IgG)	89.0	94.1	93.0	89.0	94.0	3
	81.0	75.0	76.0	80.0	78.0	5
	80.0	80.0	69.0	88.0	80.0	6
	66.0	66.6	66.0	66.6	66.3	Current study
Pylori Stat (Bio-Whittaker, Maryland) (serum IgG)	96.3	93.9	89.7	97.9	94.7	8
	97.9	48.1	63.5	96.1	72.0	Current study

Ig Immunoglobulin

TABLE 2
Medication use in the study population

	Helicobacter pylori status (%)*		
Medications	**Positive n=50 (%)**	**Negative n=54 (%)**	**P**
Antibiotic use in month before biopsy (n)†:	48	51	
None	44 (91.6)	39 (76.4)	
Active against *H pylori*	1 (2.1)	6 (11.8)	0.09‡
Not active against *H pylori*	3 (6.3)	6 (11.8)	
Number of antiulcer medications being taken in month before biopsy (n):	48	52	
None	17 (35.4)	17 (32.7)	
One	18 (37.5)	25 (48.1)	0.68‡
Two	11 (22.9)	9 (17.3)	
Three or more	2 (4.2)	1 (1.9)	
Number of patients taking bismuth-containing compounds	5 (10.4)	0	0.017
Number of patients taking omeprazole	5 (10.4)	8 (15.4)	0.46‡
Number of patients taking omeprazole and/or bismuth-containing compounds	7 (14.6)	8 (15.4)	0.9‡
Number of patients not taking ASA or an NSAID	13 (26.0)	13 (24.1)	0.34‡
Number of patients with symptoms of peptic ulcer disease	50 (100)	47 (87)	0.03
Number of patients with endoscopically proven peptic ulcer disease	16 (32)	7 (13)	0.02

*H pylori *status as determined by histology. †Data concerning antibiotic and antiulcer medication use were not available for all patients, n is the number for whom data were available. ‡Not statistically significant. ASA Acetyl salicylic acid; NSAID Nonsteroidal anti-inflammatory drug*

without *H pylori* infection in terms of number of antiulcer medications used, or use of acetylsalicyclic acid or nonsteroidal anti-inflammatory drugs. Antibiotic use approached statistical significance in those who were infected by *H pylori*. Of the patients with positive histology, a significantly greater proportion (five patients, P=0.017) were taking bismuth-containing compounds. The use of bismuth-containing compounds or omeprazole did not alter the histolopathology results. Patients histologically positive for *H pylori* were significantly more likely to have dyspeptic symptoms and epigastric pain (P=0.034), as well as endoscopic findings compatible with *H pylori*-induced disease (P=0.023).

DISCUSSION

Since the development of the EIA, this assay has been adopted to measure the immune response to a variety of different infecting agents. A prime example of the versatility of this technique is how it has been modified to measure immunoglobulins in saliva as in the case of *H pylori*. Table 1 demonstrates our study results and the published characteristics of commercially available noninvasive *H pylori* detection assays. The reported test accuracies vary greatly.

The salivary EIA has been proposed as a safe, noninvasive and reliable technique for diagnosing and following the response to treatment of *H pylori* infection (3); however, in our hands, this technique had poor accuracy and as such, is not suitable for wide scale use. The problem that plagues the diagnosis of *H pylori* infection is the absence of a true gold standard against which diagnostic tests can be evaluated (10,11). Histological examinations of endoscopically obtained tissue had been proposed as the gold standard (10,11); however there may be an underdetection of *H pylori* infection due to sampling and the patchy nature of the disease (10), the histological staining techniques used and the experience of the pathologist interpreting the slides (12). Furthermore, histology and culture both require endoscopy.

Alternatively, the urea breath test may be used to test for *H pylori* because it compares favourably with histology (7). Both of these investigations are expensive and may not be routinely available in smaller communities. The Pylori Stat Serum IgG EIA had an excellent sensitivity of 97.9%; however, its specificity was poor at 48.1%. There are a number of possible explanations for this low specificity: crossreaction with antigens from other bacteria (12); eradication of the organism

from antimicrobial use, leading to negative histology while serology remains positive (12); and patchy involvement of mucosa and sampling of a noninvolved area (12). The Helisal Salivary IgG EIA lacked both sensitivity (66.0%) and specificity (66.6%). This test relies upon salivary IgG that is derived from serum and is present at concentrations of 1/900 of that in serum (13) and as such may be present at a concentration below the detectable level. Salivary IgG may also be more susceptible to fluctuations from therapy than serum IgG determinations. As suggested by the manufacturer, we kept the saliva specimens at room temperature until they were ready for processing. Salivary proteolytic enzymes may have destroyed the immunoglobulins. All of these factors may have contributed to the poor sensitivity and specificity of this test. Another factor that may have influenced our results is the selection of our gold standard. Some investigators have used culture and/or histopathological examinations (sensitivity of 81%) (5), histology (sensitivity ranging from 80% to 96%) (6,12), serum EIA (4), histology or urease test (2), while others have redefined the optical density cut-off for EIA positivity to maximize diagnostic accuracy (4). Of note, a group of investigators was unable to augment diagnostic accuracy, even with manipulation of EIA cut off values (6). Culture alone does not appear to be a suitable gold standard because of its high false negative rate (10). Our results are most compatible with those of studies using histopathology as the gold standard (5,6,12). Serology, unfortunately, gives a poor indication of a patient's current infection status, particularly in patients who have received or who are currently receiving antimicrobials or proton pump inhibitors. These patients may be histopathologically and culture negative for *H pylori,* yet remain seropositive (14).

As anticipated by the poor sensitivity and specificity values, the PPV (66.0%) and NPV (66.6%) were low, thus reducing the utility of this easily administered, noninvasive diagnostic test. Based upon our findings the salivary IgG EIA does not appear to be a suitable assay for diagnosing *H pylori* infection. This is disappointing because of its ease of sample collection and noninvasive nature. A salivary collection technique would be ideal for use in screening children and in remote communities. The serum IgG EIA is a reasonable screening tool before endoscopy due to its high NPV. It may thus be possible to decrease the number of endoscopies necessary by screening dyspeptic patients (15).

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