Validation of a new saliva test for *Helicobacter pylori* infection

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OF BATHE, AJ RAE, P ZETLER, IGM CLEATOR. Validation of a new saliva test for *Helicobacter pylori* infection. Can J Gastroenterol 1996;10(2):93-96. The purpose of this study was to evaluate a recently introduced saliva test measuring immunoglobulin (Ig) G antibodies to *Helicobacter pylori* by enzyme-linked immunosorbent assay (ELISA). ELISA has previously been validated against IgG serological tests; however, it is not considered the definitive test for *H pylori* infection. Using endoscopic antral biopsies as the 'gold standard' for comparison, the saliva test was validated on 70 patients with upper gastrointestinal symptoms admitted to St Paul's Hospital Gastroenterology Clinic for gastroduodenoscopy. Thirty-five patients (50%) had histological evidence of gastritis and, by using acridine orange stain, the bacterium was visualized in 25 patients (71%). A biopsy was considered positive when the bacterium was visualized. The saliva test was determined to be 80% sensitive and 80% specific for *H pylori* infection when the cut-off for a positive test was 0.3 ELISA U/mL. Positive and negative predictive values were 69% and 88%, respectively. More study is required to assess the clinical utility of the test.

Key Words: *Diagnostic test, Enzyme-linked immunosorbent assay (ELISA),* Helicobacter pylori, *Immunoglobulin G, Saliva*

Validation d'un nouveau test salivaire pour le dépistage de l'infection à *H. pylori*

RÉSUMÉ : Le but de cette étude était d'évaluer un nouveau test salivaire pour la mesure des anticorps anti-IgG dirigés contre *Helicobacter pylori* au moyen d'un dosage ELISA. Le test ELISA avait au préalable été validé dans le cas de spécimens d'IgG sérologiques. Il n'est toutefois pas jugé définitif dans le cas de l'infection à *H. pylori*. À l'aide de biopsies endoscopiques antrales servant de mesures de référence, le test salivaire a été validé auprès de 70 patients atteints de symptômes au niveau des voies digestives hautes et admis à la clinique de gastro-entérologie de l'hôpital Saint-Paul pour gastroduodénoscopie. Trente-cinq patients (50 %) manifestaient des signes histologiques de gastrite et à l'aide d'un test à l'acridine orangé, la bactérie a pu être visualisée chez 25 patients (71 %). La biopsie était jugée positive lorsque la bactérie était visualisée. Le test salivaire a été jugé sensible à 80 % et spécifique à 80 % pour l'infection à *H. pylori* lorsque le seuil fixé pour une réponse positive au test ELISA était à 0,3 U/mL. Les valeurs prédictives positives et négatives ont été respectivement de 69 % et 88 %. Il faudra approfondir la recherche si l'on veut confirmer l'utilité clinique du test.

Helicobacter pylori causes type B gastritis, a risk factor for peptic ulcer disease. The presence of the bacterium, which is found specifically on gastric epithelial cells, is very strongly associated with gastric and duodenal ulcers. The bacterium is found in close to 100% of patients with benign gastric and duodenal ulcers not associated with nonsteroidal anti-inflammatory drug (NSAID) use (1,2). Further, eradication of the organism results in a markedly increased cure rate of gastric and duodenal ulcers (3). Identification of individuals infected with *H pylori* is therefore becoming increasingly more important, especially in those with upper gastrointestinal symptoms.

Diagnosis of *H pylori* infection requires endoscopic biopsies of the antrum, which typically reveal the presence of neutrophils in the gastric epithelium. Occasionally, microabscesses are seen and the organism can be visualized with special stains. Because of the patchy distribution of the gastric inflammation and the appearance of the organism, several biopsies are usually required. While endoscopic diagnosis is considered to be the 'gold standard' and remains the mainstay of initial assessment of the patient with upper gastrointestinal symptoms, this diagnostic modality is invasive and not without complications. Recently, a saliva test measuring immunoglobulin (Ig) G antibodies to *H pylori* has been developed. This has been compared with serological tests (4-6) and with antral biopsies (5-8) in several popula-

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TABLE 1
Comparison of diagnosis of *Helicobacter pylori* infection by histological examination of gastric mucosa versus saliva test when the cut-off value for a positive test is 0.30 ELISA U/mL

	Antral biopsies		
	H pylori present	*H pylori* absent	Total
Saliva positive	20	9	29
Saliva negative	5	36	41
Total	25	45	

Sensitivity was 80%, specificity was 80%, positive predictive value was 69%, negative predictive value was 88% and accuracy was 80%. ELISA Enzyme-linked immunosorbent assay

tions. In the present study, we validated the saliva test, using antral biopsies as the gold standard of diagnosis.

PATIENTS AND METHODS

Seventy patients with upper gastrointestinal symptoms admitted to St Paul's Hospital for gastroduodenoscopy from October 1993 to September 1994 were evaluated. Forty-three females and 27 males (average age 50 years, range 20 to 77) were studied. Patients with a coagulopathy, active bleeding or other contraindications to gastric biopsy (eg, no consent) were excluded from the study. Further, patients who had been treated for *H pylori* infection within three months of the study were excluded because anti-*H pylori* antibody titres may remain elevated for a variable period following eradication of the organism (2).

Saliva serology: Using the Omni-SAL sterile saliva collection device (Saliva Diagnostic Systems, Inc, Oregon), an unstimulated saliva sample was obtained just before endoscopy. The saliva collection device consists of an absorbable pad connected to an indicator confirming the adequacy of the saliva collection. The indicator turns blue when an adequate quantity of saliva has been collected; this occurred within 1 to 5 mins of inserting the collection device in the mouth. Several patients could not produce enough saliva to complete the test and were excluded from the study. Saliva samples were refrigerated until the IgG antibody to *H pylori* in the saliva was measured by an enzyme-linked immunosorbent assay (ELISA) according to instructions from the manufacturer (Cortecs Diagnostics, London, United Kingdom) by technicians blinded to patients' conditions and biopsy results. Standards were run with each batch of samples tested. Antibody levels were expressed as ELISA units, arbitrary units of optical density defined by Cortecs Diagnostics. The saliva assay was considered positive if the antibody levels were greater than 0.3 ELISA U/mL, negative if antibody levels were less than 0.25 ELISA U/mL and equivocal if antibody levels were between 0.25 and 0.3 ELISA U/mL, as suggested by the manufacturer when this study was initiated.

To test the reproducibility of the saliva test, 37 patients and eight asymptomatic volunteers performed the test twice on the same day. To validate the test, the first saliva test submitted in patients who performed the test twice was compared with the antral mucosal biopsy.

Pathology: Two antral biopsies were taken from each patient. Biopsies were stained with hematoxylin and eosin for conventional histology and with acridine orange for detection of *H pylori*. Biopsies were examined by a pathologist who was blinded to the clinical status of the patient and other test results. Scores of the mononuclear and neutrophilic cell densities (range 0 to 3) were obtained separately in accordance with the Sydney scoring system for gastritis (9). Similarly, the number of bacteria was graded (range 0 to 3). A classification of mild infestation (grade 1) represented occasional organisms in occasional crypts, moderate (grade 2) denoted many crypts showing organisms, and severe (grade 3) implied that the majority of crypts and most of the surface showed innumerable organisms. Patchiness of distribution and size of organisms led the authors to classify as mild, moderate or severe rather than by density. Only biopsies in which *H pylori* was visualized were considered positive.

Statistical analysis: The strength of the relationship of antibody levels with various histological parameters was determined by calculation of Spearman's rank correlation (rho). A *t* test for correlation was used to test the significance of the relationship between antibody levels and histological parameters. P values were subjected to Bonferroni correction.

RESULTS

Validation of saliva test: Thirty-five patients (50%) had histological evidence of acute or chronic gastritis and 25 patients (36%) had *H pylori* infections as diagnosed on biopsy. All patients whose antral biopsies showed *H pylori* had histological gastritis, whereas only 10 of 45 patients (22%) without infection had gastritis.

A comparison of the saliva test with antral biopsies is summarized in Table 1. None of the patients tested had antibody levels in the 'equivocal' range. The saliva test was determined to be 80% sensitive and 80% specific for *H pylori* infection. Positive and negative predictive values were 69% and 88%, respectively.

To determine whether decreasing the cut-off value for a positive test improves the sensitivity, a receiver operating characteristic curve was plotted (Figure 1). These curves are particularly valuable for evaluating the degree of specificity lost for each decrease in the cut-off value for a positive test. Analysis of the curve shows that, with decreases in the cut-off value for a positive test, the sensitivity increases minimally, to a maximum sensitivity of 84%. To achieve such a sensitivity, however, a large price is paid in that the number of false positives obtained increases dramatically.

Two of the eight asymptomatic volunteers (25%) who performed the saliva test twice had positive initial results. The reproducibility of the saliva test in this group was 100%. Of the 37 patients who performed the test twice, 23 (62%) were positive on the first test. Four patients had different results on testing a second time. The reproducibility of the saliva test in patients was 89%. The overall reproducibility (in patients and asymptomatic volunteers) was 91%.

Relationship of histological grade with antibody levels: The relationship of antibody levels with the degrees of severity of the histological parameters under study is shown in Figure 2.

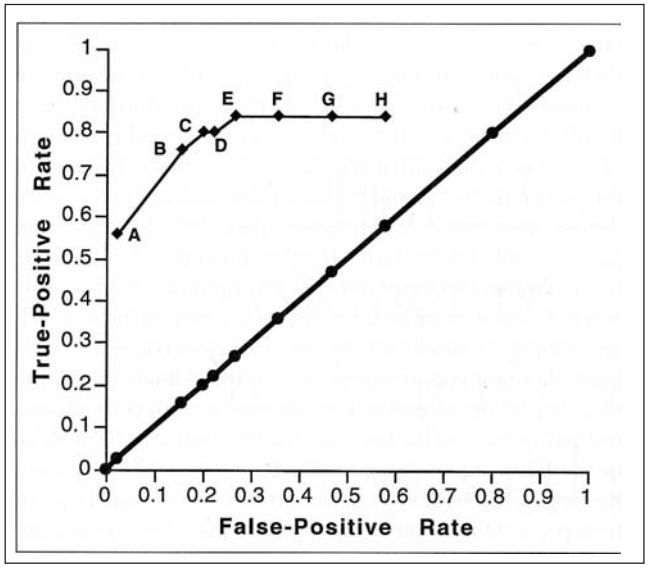

Figure 1) *Receiver operating characteristic curve for saliva test. Key cut-off values are: A=1.0, B=0.4, C=0.3, D=0.25, E=0.2, F=0.15, G=0.10, H=0.05. The straight line represents a hypothetical test that yields a positive or negative result by chance*

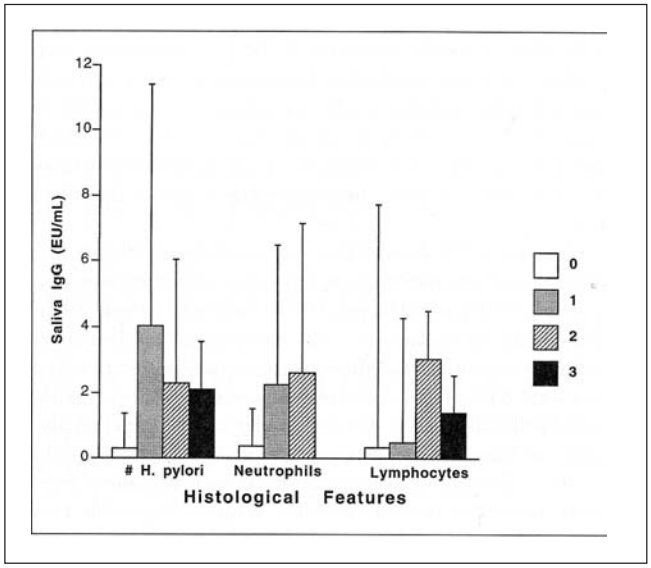

Figure 2) *Relationship of antibody levels (as reflected by optical density) and grades of histopathological parameters studied. Error bars represent standard deviation. EU Enzyme-linked immunosorbent assay units; H pylori Helicobacter pylori; Ig Immunoglobulin*

The Spearman correlation coefficients of antibody levels with degrees of bacterial, neutrophil and lymphocyte infiltration were 0.58, 0.56 and 0.54, respectively. There was a significant nonparametric correlation ($P<0.001$) between antibody levels and severity of each of the histological characteristics under study.

DISCUSSION

In an attempt to obviate the risks and inconvenience of current methods to diagnose *H pylori* infection, numerous noninvasive tests for *H pylori* infection are being developed. The saliva test for anti-*H pylori* IgG is extremely easy to administer. The assay is based on the presence of *H pylori*-specific immunoglobulins in saliva. The salivary glands produce mainly IgA, whereas IgG and IgM are mainly derived by transudation from blood to gingival fluid (10). When IgG serological tests for *H pylori* were used as a gold standard for comparison, the saliva assay used in this study was reported to be greater than 90% sensitive and specific (4). There are several problems with this comparison, however. The main criticism is that the serology tests have been validated by comparison with gastric mucosal biopsies as the gold standard, which shows serology to be greater than 90% accurate in the diagnosis of *H pylori* infection (2,5,11). The second problem with comparing the saliva test with serology is that the two tests presumably measure the same parameter: the presence of anti-*H pylori* immunoglobulins. While this comparison is necessary to define the most effective method of measuring the antibodies, the limitations of using antibody titres to make a diagnosis must be kept in mind. The major limitation is the lag time between the appearance and disappearance of IgG antibodies after infection or eradication of *H pylori*. Following infection with *H pylori*, antibody titres may take several months to become elevated. Further, antibodies may remain elevated for up to 12 months following eradication of the organism (2). Using serology as a gold standard with which other tests are compared is therefore of limited value.

The choice of test for the gold standard for *H pylori* infection has generated much controversy. While antral biopsies for histological examination may be inaccurate because of the patchy nature of *H pylori* infection, a recent study comparing seven invasive and noninvasive tests for infection showed that this was the most sensitive and specific method of diagnosis available (11). IgG serology is also very sensitive, but has limitations, as mentioned above. The ^{13}C-urea breath test is greater than 90% sensitive and specific, and this may have been a good test to use in combination with antral biopsies in the present study. Because antral biopsies and the ^{13}C-urea breath test each have positive predictive values greater than 97% (11), this may have improved our positive predictive value, for only 71% of the patients with gastritis had *H pylori* detected. On the other hand, it is possible that many of the negative biopsies may represent so-called reactive gastritis, occurring in response to agents causing mucosal surface damage (eg, alcohol, bile reflux, NSAIDs) and not *H pylori* gastritis. Results from other investigators seem to vary based on the gold standard(s) used for comparison. Lin et al (8) used antral biopsies as the gold standard, and the sensitivity, specificity, and positive and negative predictive values were 81%, 72%, 67% and 84%, respectively. These results corresponded quite closely to our findings. Nilius et al (6), using antral histology and a urease (cod liver oil) test as the gold standard, reported a sensitivity of 57%, a specificity of 87%, and positive and negative predictive values of 87% and 56%. Luzza and co-workers (5), using the same two gold standards, observed a sensitivity, specificity, positive and negative predictive values of 82%,

71%, 95% and 40%, respectively. Each of these latter two studies observed considerably higher positive predictive values and lower negative predictive values than our results or those of Lin et al (8). Use of more than one test with a high sensitivity and positive predictive value may therefore have yielded a more accurate measure of the utility of the saliva test.

Patel et al (7) showed that measurement of salivary IgG distinguished positive from negative cases of *H pyl*ori infection as diagnosed by antral biopsy, but salivary IgA did not. When the salivary IgG assay was optimized by adjusting the cut-off value for a positive test, the sensitivity and specificity of the test were 85%. The poor performance of salivary IgA compared with salivary IgG was expected because serum IgA also tends to have a lower antigen specificity than serum IgG (2,5,11). We could not demonstrate such accuracy, even with changes in the cut-off value. While it is possible that this is because of the limited sensitivity of biopsies in detecting *H pylori* infection, analysis using the receiver operating characteristic curve suggests another explanation. There appears to be an intrinsic limitation in test sensitivity. One possibility is that the limitation in sensitivity is related to the nature of the antigen comprising the ELISA, although the antigen is chromatographically purified, as are the most sensitive and specific commercially available serological tests. On the other hand, the antibody levels in the saliva (particularly those of the IgG variant) may be too low in some individuals with an *H pylori* infection.

The value of noninvasive tests for *H pylori* infection must continue to be evaluated as their accuracies and ease of use continue to improve and as disparate results appear in the literature. While Patel and colleagues (7) demonstrated that 39% fewer endoscopies had to be performed when the saliva assay and a history of NSAID use were used to screen patients under 45 years of age, Luzza et al (5) showed the saliva test could not discriminate patients with from those without duodenal ulcers. In addition, while we showed a correlation between anti-*H pylori* IgG levels as measured by saliva test and the degree of severity of gastritis and bacterial infiltrate, Figure 2 shows that extremely variable results can be obtained under a given condition. The saliva test results are therefore quite unreliable as an index of the severity of gastroduodenal disease. We believe that gastroduodenoscopy is still the diagnostic test of choice for the initial evaluation of upper gastrointestinal symptoms. Such symptoms are notoriously nonspecific and may represent gastritis, peptic ulcer disease, malignancy or extragastric pathology. Identification of individuals infected with *H pylori* does not aid in defining the pathology responsible for the symptoms, and the only way to rule out malignancy is by examination of the stomach and biopsy of suspicious lesions. Noninvasive tests for *H pylori* should therefore not be used as the primary diagnostic modality in the assessment of patients with upper gastrointestinal symptoms. Rather, noninvasive tests may be of value in the follow-up of patients who have received eradication therapy. That is, following administration of anti-*H pylori* therapy, confirmation of eradication may best be accomplished using a noninvasive test, thereby obviating a second endoscopic procedure.

Clearly, use of noninvasive tests must be delineated according to clinical indication. Our experience suggests that the ^{14}C-urea breath test is the noninvasive test of choice at our institution (12). Re-testing must be performed no sooner than one month after therapy has been completed. It can be performed in a short time, and because the correlation between cumulative 60 and 10 min collection is excellent and does not compromise test accuracy, patient acceptance is likely to be improved. Remote centres have also been performing these tests and sending them to our centre for analysis – a further argument for the ease of use of this test, even in poorly equipped centres. As for blood tests, vast improvements in reliability still are overshadowed by the dilemma of seroconversion. In other words, how long after anti-*H pylori* therapy should the test be administered to check for eradication?

In its present form, the saliva test has been shown to be ineffective for screening patients or for follow-up confirmation of eradication. ^{13}C- and ^{14}C-breath tests and serology tests have far superior sensitivities and specificities. Several studies cited in our paper corroborate our results.

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