Evaluation of salivary antibodies to detect infection with Helicobacter pylori

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Helicobacter pylori infection is an important cause of peptic ulcer disease and chronic gastritis (1). Infection with this bacterium stimulates the production of immunoglobulin (Ig) G antibody. Salivary IgG antibody tests to detect H pylori infection offer a convenient and noninvasive method of diagnosis. To evaluate an IgG salivary antibody kit, saliva was collected from 157 out-patients with dyspepsia referred for endoscopy to a tertiary centre. A salivary IgG ELISA antibody assay was performed using the Helisal Helicobacter pylori (IgG) assay kit, and at least four gastric biopsies were obtained. H pylori infection was confirmed by demonstration of the organism on Warthin-Starry silver stain (sensitivity 85%, specificity 55%). The prevalence of infection with H pylori was 30%. When the analysis was redone, excluding those treated with eradication therapy, the results were similar (sensitivity 86%, specificity 58%). The positive predictive value of the assay was 45% and the negative predictive value was 90%. Despite the ease of sampling, the assay used has limited diagnostic utility, lacking the predictive value to indicate which patients referred with dyspeptic symptoms to a tertiary care setting are infected with H pylori.

Key Words: Diagnostic test, Helicobacter pylori, Salivary antibodies

Utilité des anticorps salivaires pour le dépistage de l’infection à Helicobacter pylori

RÉSUMÉ : L’infection à Helicobacter pylori est une importante cause de l’ulcère gastro-duodénal et de la gastrite chronique. Cette infection bactérienne favorise la production de gammaglobulines (IgG). Les tests de dépistage des IgG salivaires appliqués au diagnostic de l’infection à H. pylori constituent une méthode non vulnérante et pratique. Des échantillons de salive de 157 patients atteints de dyspepsie, non hospitalisés mais adressés pour endoscopie dans un centre de soins tertiaires, ont été recueillis dans le but d’évaluer une troume de dépistage des IgG. Un dosage des IgG par méthode ELISA a été effectué au moyen de la trousse Helisal Helicobacter pylori IgG et au moins quatre biopsies gastriques ont été obtenues. L’infection à H. pylori a été confirmée au moyen de la coloration de Warthin-Starry (sensibilité 85 % et spécificité 55 %). La prévalence de l’infection à H. pylori a été évaluée à 30 %. Lorsque l’analyse a été répétée en excluant les patients ayant subi un traitement d’éradication, les résultats ont été semblables (sensibilité 86 % et spécificité 58 %). La valeur prédictive positive du dosage a été de 45 %, la valeur prédictive négative, de 90 %. Malgré la facilité du prélèvement des échantillons, le dosage possède une utilité diagnostique limitée, puisqu’il ne permet pas de prédire quels patients adressés dans un centre de soins tertiaires pour symptômes dyspeptiques risquent d’être infectés par H. pylori.
numbers of organisms. Patient's remaining gastric biopsies and repeated in inflamed silver stains were negative, additional stains were done on that a pathologist who was blinded to the serological result. If the organism on Warthin-Starry silver stain of the antral biopsies by

A total of 157 consecutive out-patients with at least a one-month history of dyspepsia, defined by upper abdominal pain or discomfort accompanied by fullness, burning, belching, nausea, vomiting, fatty food intolerance or difficulty completing a meal, referred to McMaster University Medical Centre, Hamilton, Ontario for endoscopy, were studied. Before endoscopy, saliva was collected from each patient using the Omni-SAL collection device (Cortecs Diagnostics, Isleworth, United Kingdom), stored at 4°C and processed within two months. Treatment with proton pump inhibitors, nonsteroidal anti-inflammatory drugs (NSAIDs) or H pylori eradication therapy was recorded. At least four gastric biopsy samples were obtained from each patient, including two from the antrum, one from the angle and one from the body of the fundus. H pylori infection was confirmed by demonstration of the organism on Warthin-Starry silver stain of the antral biopsies by a pathologist who was blinded to the serological result. If the silver stains were negative, additional stains were done on that patient’s remaining gastric biopsies and repeated in inflamed antral biopsies to reduce the chances of failing to detect scant numbers of organisms.

The Helisal Helicobacter pylori (IgG) assay kit (Cortecs Diagnostics), an ELISA, was used to measure salivary antibodies. The assay was used as specified by the manufacturer with no modifications. First, 100 µL of saliva was added to each well of a 96-microwell plate coated with H pylori antigen. Following 30 mins of incubation at room temperature wells were washed five times using an automated plate washer. Then 50 µL of anti-human biotinylate (rabbit anti-human IgG antibody conjugated to biotin) was added to each well and allowed to incubate at room temperature for 30 mins. After five washes, 50 µL of avidin peroxidase conjugate (horseradish peroxidase conjugated streptavidin) was added to each well and allowed to incubate for 15 mins. Following another five washes, 100 µL of substrate solution (1:1 mixture of tetramethylbenzidine and hydrogen peroxide) was added. After 30 mins, 50 µL of stop solution (1 M sulphuric acid) was added to each well. Absorbance was read at 450 nm by a spectrophotometer. A standard curve was constructed during each assay using the supplied standard solution. Borderline, positive and negative controls supplied by the manufacturer were run with each assay. Samples with ELISA units of at least 1.00 were considered positive for salivary H pylori antibody, samples of less than 0.80 were considered negative and those between 0.80 and 0.99 were equivocal, as recommended by the manufacturer.

### RESULTS

Of the 157 patients (mean age 44 years, range 17 to 75; 68 males), 47 (30%) were infected with H pylori, as diagnosed by a positive biopsy. The sensitivity of the Helisal Helicobacter pylori (IgG) assay was 85% (40 of 47) and the specificity 55% (61 of 110). Eighty-eight patients (56%) had a pathological diagnosis of gastritis, six (4%) of gastric ulcers and nine (6%) of duodenal ulcers (Table 1). Of the six patients with gastric ulcers, three had H pylori detected on biopsy; two others were on NSAIDs in the month before endoscopy and one received eradication therapy in the year before endoscopy. Of the nine patients with duodenal ulcers, three had H pylori detected on biopsy, while five who were biopsy negative received eradication therapy before endoscopy.

| TABLE 1 | Diagnostic groupings based on Helicobacter pylori histological status and salivary antibody results |
|-----------------|----------------|----------|----------|----------|----------|
| H pylori status | Gastric ulcer | Duodenal ulcer | Other* | None | # (%) of patients with histologic gastritis |
| H pylori biopsy positive (n=47) | 3 (6) | 3 (6) | 26 (55) | 15 (32) | 45 (96) |
| Positive salivary antibody (n=40) | 3 (8) | 2 (5) | 22 (55) | 13 (33) | 40 (100) |
| H pylori negative (n=110) | 3 (3) | 6 (5) | 50 (45) | 51 (46) | 43 (39) |
| Positive salivary antibody (n=49) | 0 (0) | 3 (6) | 29 (59) | 17 (35) | 4 (8) |

*Includes patients with esophagitis, duodenitis, gastritis, hiatus hernia and chemical gastropathy

| TABLE 2 | Treatment characteristics of patients |
|-----------------|----------------|----------|----------|
| H pylori status | Eradication therapy within 12 months before endoscopy | Hydrogen ion pump inhibitors within one month of endoscopy | NSAIDs within one month of endoscopy |
| Histology + / Antibody + (n=40) | 2 (5) | 6 (15) | 2 (5) |
| Histology + / Antibody – (n=7) | 1 (3) | 1 (3) | 0 (0) |
| Histology – / Antibody + (n=49) | 9 (18) | 11 (22) | 4 (8) |
| Histology – / Antibody – (n=61) | 6 (10) | 14 (23) | 5 (8) |

NSAID Nonsteroidal anti-inflammatory drug

has been observed to be a convenient, noninvasive method to detect infection. The objective of this study was to evaluate the use of salivary IgG antibodies to detect H pylori infection in patients referred with dyspepsia who underwent endoscopy.
Salivary antibodies to *Helicobacter pylori*

Forty-five of the 49 salivary positive-biopsy negative patients had no or minimal chronic inflammation on biopsy. Four had inflammation present. Nine of the 49 patients (18%) versus two (5%) salivary positive-biopsy positive patients had received eradication antibiotic therapy in the 12 months before biopsy. Also, 11 of the 49 patients (22%), compared with six (15%) salivary positive-biopsy positive patients, had been on hydrogen ion pump inhibitors in the month before endoscopy (Table 2). *H pylori* was detected in the gastric body of only four of 53 salivary positive-antral biopsy negative cases.

**DISCUSSION**

A number of published studies have reported the use of salivary antibodies to detect *H pylori* infection, with sensitivity ranging from 66% to 89% and specificity from 71% to 94% (10-16). Patel et al (10) studied 119 consecutive dyspeptic patients referred for endoscopy and determined the sensitivity and specificity of both to be 85% with a salivary IgG ELISA (an adaptation of the Helico-G serum ELISA kit), compared with microscopy and the urease test. The Helisal *Helicobacter pylori* (IgG) assay was evaluated in 79 patients undergoing routine endoscopy by Moayyedi and co-workers (11). Sensitivity of 82% and specificity of 92% was noted when the assay was compared with a ‘gold standard’ that included histology, culture and a rapid urease test. Similar results were noted by Clancy et al (12) (sensitivity 89%, specificity 94%) when the Helisal *Helicobacter pylori* (IgG) assay was compared with either biopsy or urease testing. Using the same reference method, Christie et al (13) noted both a lower sensitivity (88%) and specificity (71%) in 86 patients. Simor et al (14), evaluating 195 patients using the Helisal *Helicobacter pylori* (IgG) assay with culture, histopathology or both as the reference method, found 81% sensitivity and 75% specificity. Similarly, Bathe and colleagues (15) noted sensitivity of 80% and specificity of 80% using histopathology as the reference method. Sensitivity of 84% and specificity of 81% were found by Fallon et al (16) when the results from the Helisal *Helicobacter pylori* (IgG) assay were compared with those from serum IgG. When these investigators, however, used gastric biopsy as the reference method, both the sensitivity and the specificity were lower (66% and 74%, respectively).

Although the sensitivity of the salivary antibody assay in our study (84%) was similar to that in previous studies, the specificity (54%) was unexpectedly low. Serum antibody levels fall slowly following successful eradication therapy with antibiotics (17). Our study, unlike at least two of the previous studies (14,15), included patients with a history of eradication therapy for *H pylori*. Of patients with salivary antibody detected, over three times as many biopsy negative versus biopsy positive had received eradication therapy in the 12 months before endoscopy (18% versus 5%) (Table 2). If the analysis is redone excluding previously treated individuals, however, the sensitivity and specificity of the salivary antibody assay change very little (86% sensitivity [38 of 44] and 58% specificity [55 of 95]). Prior antibiotic treatment is therefore not a likely explanation for the low specificity of the assay.

Another explanation for the low specificity may be that the salivary positive-biopsy negative individuals achieved only partial eradication, reducing demonstrable organisms but maintaining seropositivity due to persistent infection. Because only four of the 49 salivary positive-biopsy negative patients had inflammation on biopsy, we believe partial eradication is an unlikely explanation.

It has been suggested that proton pump inhibitors may cause *H pylori* to migrate from the antrum to the fundus (18), with subsequent reporting of a false negative if only the antral biopsy is examined. In our study, because the proportion of salivary positive-biopsy negative patients who were on proton pump inhibitors was comparable to that of salivary positive-biopsy positive individuals on the drug (22% versus 15%), this is unlikely. Detection of *H pylori* in the gastric body of only four of 57 salivary antibody positive-antral biopsy negative patients is further evidence that migration was not a major contributing factor to our high false positive rate.

Using biopsy alone (without culture or rapid urease test) may have resulted in underestimation of the number of true positives. The patchy nature of *H pylori* infection may have accounted for false positive serology despite that multiple biopsies were examined (19). However, the absence of chronic inflammation on biopsy in the majority of the false positive patients (45 of 49, 92%) argues against this having a significant effect.

The Helisal *Helicobacter pylori* (IgG) assay used in this study was compared with gastric biopsy alone, not with a serum antibody assay. Several studies have confirmed the accuracy of serum antibody assays to detect *H pylori* infection (8,9,20-22). A serological assay to evaluate patients who were histology negative and had received eradication therapy would have been helpful in this study. The majority of such individuals, however, in this study did not have histological evidence of chronic inflammation, making it unlikely that they were infected.

The positive predictive value of the assay in this population of patients referred for endoscopy, with a prevalence of infection of 30%, is only 45% (40 of 89); the negative predictive value is 90% (61 of 68). Results of this study suggest that, compared with the many accurate serological assays available, the present form of the Helisal *Helicobacter pylori* (IgG) ELISA antibody assay is limited. It should be noted that these results apply in the setting of a referred tertiary centre where patients are often treated for *H pylori* infection before referral. In conclusion, despite the ease of sampling, the Helisal *Helicobacter pylori* (IgG) assay has limited diagnostic utility, lacking the predictive value to indicate which patients referred with dyspeptic symptoms to a tertiary care setting are infected with *H pylori*.

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REFERENCES
