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Diagnosis of Helicobacter pylori infection among patients with dental caries by stool antigen test

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Helicobacter pylori is the causative agent of gastritis, peptic ulcer disease and a risk factor in the development of gastric cancer.[1] The route of transmission of *H. pylori* is currently under debate, although evidence suggests that it is predominantly by direct person-to-person contact. Transmission routes vary between the developed world and developing countries. In developed countries, transmission is largely by the oral-oral route, whereas in developing countries it is by the faecal-oral route. It is also suggested that *H. pylori* exists in the natural environment.[2]

As the oral cavity is a possible reservoir for the organism, it provides a possible tool for the rapid and non-invasive diagnosis of infection. Current studies indicate that *H. pylori* is present in dental plaque, although low numbers have been reported in individual samples and numbers vary between sites in the mouth.[3]

The presence of the organisms in plaque may be intermittent, perhaps as a result of gastro-oesophageal reflux.[3,4] In addition, there is some controversy about whether or not dental plaque is a significant source for re-infection of the gastric mucosa in patients with fair to poor oral hygiene.[3]

Nasrolahei *et al*.[5] showed no significant association between *H. pylori* colonisation in dental plaque and gastric infection. Matsuda and Morizane[6] screened for the risk of acquiring *H. pylori* infection among dental professionals and non-dental professionals, and showed that the former group was at greater risk of infection. Al-Hawajri *et al*.[7] concluded that dental plaque may be a candidate reservoir for *H. pylori*, that medical equipment may contribute to *H. pylori* transmission and that sample collection techniques can bias the true prevalence of *H. pylori* in a population.

During recent years, non-invasive methods to detect *H. pylori* infection have gained importance. Current guidelines that recommend *H. pylori* eradication treatment without performing endoscopy in certain patients highlights the importance of non-invasive tests.

The stool antigen test allows sensitive and specific non-invasive detection of *H. pylori*, is cost effective and has been used in both the diagnosis of infection and to confirm *H. pylori* eradication after treatment. In Nigeria, where loss of power is frequent and *H. pylori* culture is difficult, such a test would help in the treatment and eradication of *H. pylori*.

This study aims to detect *H. pylori* in dental plaque and in gastric biopsies from patients with a range of dental problems, and to correlate results with a stool antigen test.

Forty-one patients (age range: 4–55 years, mean: 30.9 years) presenting with a range of dental problems had stool samples screened for *H. pylori* infection using a stool antigen test (Dako). Gastric biopsies were taken after the patients gave informed consent. All the patients had not been on any medication. Biopsy samples were also screened for *H. pylori* using the CLO test and culture. Dental plaque was screened for rapid urease production using the CLO test and was also cultured for *H. pylori*.

Plaque samples obtained from teeth cavities were placed in sterile bottles containing tryptone soya broth for culture and also directly inoculated into a CLO test kit for detection of rapid urease production.

Two biopsy samples were obtained from each patient: one was cultured using Dent's medium in a candle extinction jar at 37°C for three to 10 days, while the second was added to CLO test medium to screen for rapid urease production.

For the stool antigen test, an enzyme-linked immunosorbent assay (ELISA) method using monoclonal antibodies for direct, non-invasive detection of *H. pylori* was employed. Briefly, the supernatant of a faecal suspension was added to the wells of the ELISA microplate, together with horseradish peroxidase (HRP)-labelled anti-*H. pylori* monoclonal antibody. Following incubation and subsequent washing, enzyme substrate (tetramethylbenzidine [TMB]) was added to each well. In this assay, HRP oxidised TMB to a blue coloured product. Addition of a stop solution produced a colour change to yellow and the intensity was measured spectrophotometrically.

Positive and negative controls were included with each test run. The positive control at an absorbance of 450/620-650 nm ($A_{450/620 \text{ to } 650}$) was >1.00 ($A_{450}$ >1.04), while the negative control at $A_{450/620\text{-}650}$ was <0.10 (A_{450} <0.14). Test results were interpreted as follows: specimens with *A* values ≥0.15 were regarded as positive for *H. pylori* antigen, while specimens with *A* values <0.15 were regarded as negative for *H. pylori* antigen.

Patients were defined as infected when positive results were obtained with the stool antigen test or culture, or when a positive CLO test was obtained on dental plaque.

Fourteen (34%) patients had peptic ulcer disease, while 27 (66%) had marginal gingivitis and were either normal or had mild gastritis. Irrespective of disease status, all patients were found to have *H. pylori* by the stool antigen test (13 [31.7%] males, 28 [68.3%] females). Culture of dental plaque detected *H. pylori* in only 5% of patients, while the CLO test was positive in 56% of cases. Culture of gastric biopsy samples showed a 10% isolation rate, while the CLO test was positive in 61% of case.

A variety of highly sensitive and specific detection methods have been evaluated for the detection of *H. pylori* infection. Invasive tests are usually associated with problems

of cost, especially in developing countries. There are several non-invasive methods (e.g., [^{14}C] urea breathe test [UBT] and serology) but UBT alone is very expensive and serological tests do not measure active infection accurately. Thus, it is imperative to find an easy, cheap and accurate non-invasive test for diagnosing *H. pylori* infection. Various studies have examined the accuracy of the stool antigen test, a non-invasive test for the diagnosis of *H. pylori*.[8–10]

The stool antigen test has long been known as a useful diagnostic method for the detection of *H. pylori*. The present study is the first of its kind in Nigeria and results show that all patients in the study who reported with various dental problems were positive for *H. pylori* infection by the stool antigen test. The presence of the organisms in plaque obtained from asymptomatic individuals might have been due to gastro-oesophageal reflux, and might serve as a source of infection or re-infection.

The rapid urease test (CLO test) showed that *H. pylori* was present in approximately 60% of individuals who reported with dental problems alone. Although culture of *H. pylori* from dental plaque and biopsy was relatively low (5% and 10%, respectively), it demonstrates the problems associated with mismanagement of the disease when culture alone is relied on in a developing environment.

A previous report from Nigeria, where *H. pylori* isolation rate was 27% by culture, corroborates this view.[11] Prevalence by serology is shown to be 85%, while by Gram stain it is 58%.[12] However, following the test-and-treat guidelines adopted in countries where *H. pylori* prevalence is >20%, the stool antigen test would be a better option in Nigeria, as it is affordable and a more reliable means of diagnosing *H. pylori* infection in asymptomatic individuals.

The results of the present study demonstrate the significance and affordability of the stool antigen test as a diagnostic tool in the absence of culture; thus, the stool antigen test would appear to be a better option for the non-invasive detection of *H. pylori* in Nigeria. Currently, further studies are underway to confirm the usefulness of the stool antigen test in the diagnosis and also the eradication of *H. pylori* after treatment.

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