Helicobacter pylori infection and dental care

P G Hardo, A Tugnait, F Hassan, D A F Lynch, A P West, N P Mapstone, P Quirke, D M Chalmers, M J Kowolik, A T R Axon

Abstract

Sixty two patients (mean age 45.6 years) were assessed for oral hygiene and periodontal disease by dental examination before endoscopy. Information about oral care, smoking, and dentures was obtained and samples of dental plaque collected. The presence of *Helicobacter pylori* in plaque as sought by culture and polymerase chain reaction (PCR), and gastric antral biopsy specimens were taken for histological examination. Although *H pylori* was detected in the antral specimens of 34 patients (54%) all of the cultures of dental plaque were negative, and PCR was only positive from the dentures of one patient. Smokers had poor oral hygiene, visited their dentist less often, and brushed their teeth less frequently. There was no correlation of *H pylori* gastritis with either dental hygiene or periodontal disease. These results suggest that dental plaque or dentures are not an important reservoir for *H pylori* and are probably not a significant factor in transmission of the organism. The conflicting results in published works may be caused by differences in sample collection, culture techniques, or oral contamination from gastric juice as a result of gastro-oesophageal reflux at the time of endoscopy.

(Gut 1995; 37: 44-46)

Keywords: *Helicobacter pylori*, dental care.

H pylori, and may act as a reservoir for the organism and (b) to determine the relation between *H pylori* gastritis and dental health.

Methods

Subjects

Patients referred to the dyspepsia clinic were asked to participate in the study. Sixty two patients, 26 (42%) women and 36 (58%) men with a mean age 45.6 years (range 20-73), took part. The aim of the study was explained to the patient and written consent obtained. The study was approved by the local ethics committee.

Oral health assessment

This was done before endoscopy. A detailed history and clinical assessment including information regarding oral care such as, teeth cleaning, number of visits to the dentist in the last 12 months, and dentures were obtained. Oral hygiene was assessed by the simplified oral hygiene index (OHI-S),¹⁴ on a scale of 0 to 6 (0-1=good oral hygiene, 1-2=moderately good, 2-3=fair, and 3-6=poor).

A general periodontal assessment was made using the community periodontal index of treatment needs (CPITN).¹⁵ These data were used to compute a single periodontal status index value for each subject and was scored: (III) moderate periodontitis, for subject with at least one CPITN sextant recording of 4, (II) mild periodontitis, for subject with at least one CPITN sextant recording of 3 but no higher, (I) gingivitis, for subject with at least one CPITN sextant recording of 2 but no higher, (0) for subject with no teeth. This conversion was performed to simplify handling of the periodontal health/disease data.

Dental plaque was sampled at the site with the deepest pocket reading and removed from the clinical site using one sterile paper point. Both subgingival and supragingival plaque (where both present) were sampled. If the patient was a denture wearer then a sample of dental plaque was taken from the impression surface of the denture and placed in the container along side the oral specimen. The plaque sample was placed in a sterile container and immediately immersed in liquid nitrogen to freeze it. Samples were stored in a frozen state until polymerase chain reaction (PCR) testing was performed.

A second dental plaque sample was removed from the same site with a sterile curette. The instrument tip was inserted to the depth of the crevice/pocket and brought up in continuous contact with the tooth surface to sample both

Departments of Gastroenterology

P G Hardo
F Hassan
D A F Lynch
D M Chalmers
A T R Axon

Periodontology

A Tugnait
M J Kowolik

Microbiology

A P West

and Pathology

N P Mapstone
P Quirke

Centre for Digestive Diseases, The General Infirmary at Leeds, Leeds

Correspondence to:
Dr P G Hardo, Department of Gastroenterology, Centre for Digestive Diseases, The General Infirmary at Leeds, Great George Street, Leeds LS1 3EX.

Accepted for publication
10 November 1994

There is a strong correlation between the presence of *Helicobacter pylori* infection and histologically confirmed gastritis, duodenitis, and duodenal ulceration; infection with the organism may also contribute to an increased risk of gastric carcinoma.^{1 2}

The reservoir of *H pylori* and its mode of transmission are unclear. A faecal-oral, oral-oral, and in developing countries a waterborne route of infection have been suggested.^{3 4} *H pylori* has been detected by various methods in dental plaque,⁵⁻¹⁰ which has led to the suggestion that dental plaque may be responsible for the transmission of the bacteria and possibly serve as a source of reinfection after eradication treatment. *H pylori* has also been isolated from saliva,¹¹ gastric juice,¹² and faeces.¹³

Dentures, in common with most other restorations, provide hard surfaces that become colonised by oral bacteria. It is not known, however, whether their presence increases the risk of infection with *H pylori*.

The aims of this study were: (a) to confirm previous reports that dental plaque harbours

subgingival and supragingival plaque. The sample was plunged into a liquid transport medium of brain-heart infusion broth and dispersed by agitating the phial. If the patient was a denture wearer then a second sample was removed from the impression surface of the denture and dispersed in a second phial of nutrient broth, which was analysed separately from the oral specimen. The dental plaque samples obtained were sent for culture and PCR amplification of *H pylori*.

Microbiology

Dental plaque taken from patients' samples were either centrifuged on arrival for five minutes then cultured, or stored at -70°C and cultured at a later date. Plates were incubated at 37°C for two to seven days in a microaerobic atmosphere. Colonies resembling *H pylori* on VCAT agar¹⁶ were picked off for Gram staining, oxidase, catalase, and urease tests.

PCR

All PCR specimens were immediately frozen in individual containers and underwent DNA extraction using a standard proteinase K, hexadecyl-trimethyl-ammonium bromide and phenol chloroform method.¹⁷ The PCR used nested primers specific for the 16S ribosomal RNA gene of *H pylori*.¹¹ Each PCR amplification was performed with a further negative control containing water, and a positive control containing 100 fg of *H pylori* DNA.

Endoscopy

Endoscopy was performed with an Olympus GIF Q20 gastroscope under intravenous sedation, endoscopic findings were recorded, and two antral biopsy specimens were then taken. Biopsy forceps were sterilised and endoscopes were disinfected between patients using an automatic washing machine (Key MED Auto-disinfector-2).

Histological examination

The two antral biopsy specimens were processed routinely, embedded in paraffin wax, and stained with haematoxylin and eosin

TABLE I Oral care and hygiene findings in all subjects

	No (%)
Brushing teeth: times per week	62
7-14	33 (85.5)
2-5	8 (13.9)
1	1 (1.6)
Number of visits to the dentist in the last 12 months	62
2	24 (38.7)
1	6 (9.7)
Less often	32 (51.6)
Dentures present	23 (37)
Oral hygiene index (OHI) of teeth	53
0-1 (good)	15 (24.5)
1-2 (moderate)	18 (34)
2-3 (fair)	15 (24.5)
3-6 (poor)	7 (17)
Periodontal status scale (PSI)	62
III=moderate periodontitis	3 (4.8)
II=mild periodontitis	16 (25.8)
I=gingivitis	34 (45.8)
0=no teeth	9 (14.5)

TABLE II Identification of *H pylori* from gastric antrum and dental plaque

	No (%)
<i>H pylori</i> in antral biopsy specimens	34/62 (54)
<i>H pylori</i> cultured from dental plaque	0/62 (0)
<i>H pylori</i> identified from dental plaque (denture) by PCR	1/62 (1.6)

and by the modified Giemsa technique to show the bacteria histologically. Changes of gastritis and *H pylori* colonisation were classified simply as present or absent.

Statistical methods

The data were analysed using SPSS for Windows statistical software (release 5.0) Spearman correlation coefficients were calculated and p values of <0.05 were regarded as being statistically significant.

Results

Table I shows the findings of the patients' oral care and hygiene status. Most (85.5%) patients said they brushed their teeth at least once a day. More than half (58.5%) of assessed patients had good to moderate OHI score. A small percentage (4.8%) had moderate periodontitis. Twenty three (37%) patients (mean age 54.5 years) had dentures, and none (14.5%) were edentulous.

The endoscopic findings were: normal appearance 24 (38.7%), duodenal ulcer 11 (17.7%), oesophagitis 11 (17.7%), hiatus hernia 11 (17.7%), gastric ulcer 3 (4.8%), Barrett's oesophagus 1 (1.6%), and gastric cancer 1 (1.6%).

H pylori was detected in antral samples from 34 patients (54%) all of whom had gastritis. None of the dental plaque cultures were positive for *H pylori*. One patient (age 70) with *H pylori* gastritis and a normal endoscopy had a positive PCR on a sample of dental plaque take from denture. All the other samples of dental plaque were negative by PCR (Table II).

Table III shows the correlation between *H pylori* in gastric antrum, smoking and with gastritis, and measures of dental health. All the antral specimens containing *H pylori* showed gastritis on histological examination. There was no significant correlation between *H pylori* gastritis and age, sex, dental hygiene, or periodontal disease. Smokers had higher scores for poor oral hygiene ($p=0.0001$), and visited their dentist less often ($p=0.001$). Smoking was not associated with a higher rate of *H pylori* gastritis.

TABLE III Correlation of *H pylori* in gastric antrum and smoking with gastritis and measures of dental health

	<i>H pylori</i> in antral biopsy specimens		Smoking (cigarettes/day)	
	r	p	r	p
Antral gastritis	1.0	0.0001	0.21	NS
Time between visits to dentist	0.18	NS	-0.41	0.001
Brushing teeth - times per week	-0.03	NS	-0.29	0.03
Oral hygiene index 0 (good)-6 (poor)	0.26	NS	0.49	0.001
Periodontal status scale	-0.05	NS	0.17	NS

Discussion

Robson and Moynihan in 1904, suggested that oral sepsis might play a part in the pathogenesis of gastric ulcers.¹⁸ Recently, attention has been focused on the importance of dental plaque in harbouring *H pylori* and its role in the epidemiology of *H pylori* infection. Published works in this field give conflicting results on the incidence of *H pylori* detected in dental plaque and its significance. Reports from India⁶ and Scotland⁵ on a small number of subjects showed a high prevalence of positive culture in dental plaque. However, successful culture elsewhere is rare.^{9,19}

Others reported negative dental plaque culture but a positive PCR reaction.⁸ PCR is a very sensitive technique but a positive result cannot confirm viability of the bacteria, nor exclude the possibility of bacterial contamination.

Our results with PCR and culture show that *H pylori* is rarely present in dental plaque in patients with *H pylori* gastritis or peptic ulcer disease. Our one positive PCR result may be explained by contamination of dentures from gastric juice as a result of gastro-oesophageal reflux.

It is difficult to explain the difference reported in published works between the high and low detection rate of *H pylori* in dental plaque. This may result from methods of sample collection and culture technique, or oral contamination caused by gastro-oesophageal reflux at the time of endoscopy. Future studies should include samples of dental plaque taken before and after endoscopy.

The abnormally high positive results from India may reflect the local standards of dental care and hygiene, eating habits, sanitation practice, and socioeconomic class.

We conclude that dental plaque is not an important reservoir for *H pylori* in the United Kingdom and cannot be a significant factor in the method of transmission of these bacteria in developed countries.

This work has been published as an abstract in *Gut* 1993; 34 (suppl 4): F277.

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