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Prevalence of *Helicobacter pylori* detected by polymerase chain reaction in the oral cavity of periodontitis patients

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Helicobacter pylori is an important gastrointestinal pathogen associated with gastritis, peptic ulcers, and an increased risk of gastric carcinoma. The oral cavity has been indicated as a possible *H. pylori* reservoir, and may therefore be involved in the reinfection of the stomach which sometimes follows treatment of *H. pylori* infection. The objective of the present study was to evaluate the prevalence of *H. pylori* as detected by polymerase chain reaction (PCR) in the oral cavity of periodontitis patients testing positive for this bacterium in the stomach. Thirty adult patients with alterations of the superior digestive tract, testing urease positive after endoscopy and biopsy, were selected. A full-mouth periodontal examination was performed in every patient and the subjects were allocated to two groups: gingivitis (15 patients) and chronic periodontitis (15 patients). Plaque and saliva samples collected from each patient were stored in 0.5 ml of TE buffer. DNA was extracted from the samples by the boiling method and was evaluated for the presence of *H. pylori* using the PCR method. JW 22/23 primers were used. The DNA of ATCC *H. pylori* 43629 (positive control) and water (negative control) were used for controlling the reactions. Of the 30 evaluated patients, 13 (43.3%) harbored *H. pylori* in the mouth. The bacterium was not found on the dorsum of the tongue of any patient, but was found in saliva in three patients (10%), in the supragingival plaque in six patients (20%), and in the subgingival plaque in eight patients (26.6%). The presence of *H. pylori* was similar in the gingivitis and chronic periodontitis groups. In conclusion, a high percentage of patients harbored *H. pylori* in their mouth. The bacterium was detected in saliva, supragingival and subgingival plaque, suggesting that these sites may be considered reservoirs for *H. pylori* in urease-positive patients.

Key words: chronic periodontitis; gingivitis; *Helicobacter pylori*; plaque; saliva

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Helicobacter pylori is a microaerophilic, spiral, gram-negative bacterium originally classified as *Campylobacter pyloridis*. It is an important gastrointestinal pathogen associated with gastritis, peptic ulcers, and an increased risk of gastric carcinoma (8, 14). *H. pylori* infection can be successfully treated with antibiotic therapy. There is an 80–90% eradication rate using triple therapy (association of two antimicrobials and proton pump inhibitor) (4). Reinfection of the stomach can be seen after treatment, and occurrence of the same clone of *H. pylori* in dental plaque and in gastric biopsy has been reported (23). *H. pylori* has been detected in saliva (9, 13, 20, 26), in supragingival plaque (3, 6, 13, 17), in subgingival plaque (22) and on the dorsum of the tongue (20). The presence of *H. pylori* in the oral cavity raised the question of whether the mouth could harbor this pathogenic organism, and whether this could be the source for reinfection of the stomach after treatment (13, 15, 29). Nevertheless, great variability in the prevalence of *H. pylori* in the oral cavitiy of diverse populations has been shown (3, 6, 15, 17, 22, 27, 29). There is a need to clarify the prevalence of *H. pylori* in different populations since the

difficulties in definitely eradicating it from the stomach may be related to its presence in the oral cavity (15). In addition to that, only Umeda et al. (29), without classifying periodontal disease, compared the prevalence of *H. pylori* in patients with and without deepened periodontal pockets and showed a higher prevalence of these bacteria in patients with deep periodontal pockets. It would be interesting to evaluate the prevalence of *H. pylori* in chronic periodontitis and gingivitis patients. The aim of this study was to evaluate the prevalence of *H. pylori* detected by polymerase chain reaction (PCR) in the oral cavity of periodontitis patients testing positive for this bacterium in the stomach.

Material and methods

Clinical procedures

A total of 213 patients from the Gastroenterology Division of the Universidade Federal de São Paulo (UNIFESP) with alterations of the superior digestive tract were evaluated. Only patients positive to urease test and presenting gingivitis or periodontitis were included in this study. Thirty dentate patients aged 21–62 years (11 male and 19 female) testing positive to urease (from antral biopsies) were selected. Subjects with diabetes, pregnant women, HIV-positive patients, and patients who had taken antisecretor or antimicrobials within the previous 2 months were excluded from the study. All clinical parameters were collected by the same investigator. The clinical parameters evaluated were probing depth, bleeding on probing, and clinical attachment level. Patients were allocated to one of two groups: gingivitis (G) or chronic periodontitis (P). Fifteen patients presenting probing depth ≤3 mm and at least four sites exhibiting bleeding on probing were included in the gingivitis group. Fifteen patients exhibiting bleeding on probing and at least four teeth with a probing depth ≥5 mm were allocated to the chronic periodontitis group. Patients with healthy gingiva and aggressive periodontitis patients were not included. Samples were collected from the oral cavity 2 weeks after the clinical periodontal examination. Unstimulated saliva samples produced for 1 min were collected. Plaque samples were collected from the tongue by scraping the posterior third of its dorsum with a sterile wood wedge. Supragingival plaque was collected from two different teeth using a periodontal curette. Subgingival plaque was obtained after removal of supragingival plaque, by inserting two sterile paper points into the pocket for 20 s. In the gingivitis group, supra- and subgingival plaque were collected from two sites with a probing depth ≤3 mm exhibiting bleeding on probing. The sites selected for sampling supra- and subgingival plaque in chronic periodontitis group exhibited bleeding on probing and a pocket probing depth ≥5 mm. After collection of oral samples, all patients underwent a second endoscopic examination and two biopsies were collected from the antrum of each subject. Each collected sample was identified and stored in polypropylene centrifuge tubes containing 0.5 ml of TE buffer each in a freezer at −20°C.

Microbiological procedures

Samples were vortexed and centrifuged for 2 min at 10 000 *g*. The supernatant was discarded and 100 µl of phosphate-buffered saline (PBS) was added to the pellet. The resulting solution was vortexed and centrifuged for 2 min at 5000 *g*. The supernatant was discarded and the pellet resuspended in sterile water. The tubes were immersed in boiling water for 10 min for cell lysis and centrifuged for 10 s at 13 000 *g* at 4°C. The supernatant containing the DNA was stored at −70°C until use.

The presence of *H. pylori* was evaluated by PCR. The primer pair used was homologous to 16S rDNA JW22/23 (5′- CGTTAGCTGCATTACTGGAGA-3′/ GAGCGCGTAGGCGGGATAGTC-3′) (20). A negative control, with no added DNA template, and a positive control, consisting of DNA of the strain *H. pylori* ATCC 43629 as a template, were used in all reactions.

The amplifying reaction of DNA in a volume of 25 µl was composed of 2.5 µl of 10 × PCR buffer (75 mM KCl, 10 mM Tris-HCl, pH 9.2, and 1.5 mM $MgCl_2$); 0.5 µl of desoxynucleotide triphosphate mixture (final concentration 0.2 mM each: dATP, dCTP, dTTP e dGTP; Pharmacia Biotech, Foster City, CA); 25 pM of each primer (Invitrogen, São Paulo, Brazil), 1.25 units of Hot MasterTaq (Eppendorff, Hamburg, Germany) and 10 µl of DNA template. The reaction was performed in a DNA thermal cycler (Gene amp PCR system 2400, Applied Biosystems, Foster City, CA). The initial denaturation step occurred at 94°C for 5 min, and was followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The products obtained from each reaction were resolved by electrophoresis in 2% agarose gels in Tris-acetate-EDTA buffer (TAE). Digital images of the ethidium bromide stained gels were obtained with the Photo PC 3100Z (Epson, Hemel Hempstead, England) (Fig. 1).

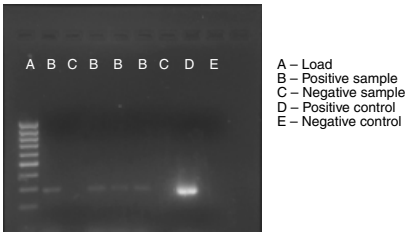

Fig. 1. Digital image of an ethidium bromide stained gel.

Statistic analysis was done using the Chi-squared test and Fisher exact test. The level of significance α was set to 5%.

Results

H. pylori were detected by PCR in all biopsy specimens obtained and in the oral cavity of 13 of 30 patients (43.3%). The organism was detected in saliva, in supra- and subgingival plaque, but not on the surface of the tongue. The distribution of the patients allocated to the gingivitis or chronic periodontitis groups according to detection of *H. pylori* in different niches in the oral cavity is shown in Tables 1 and 2. Three patients had *H. pylori* in saliva, six in supragingival plaque and eight in subgingival plaque. One of these patients was positive for *H. pylori* in saliva, in supragingival plaque of the two evaluated sites and in subgingival plaque of one site. The other patients were positive in only one of the evaluated sites. There was no statistically significant association between periodontal status (gingivitis or chronic periodontitis) and the presence of *H. pylori* in the mouth ($P = 1.0$), saliva ($P = 1.0$), supragingival ($P = 1.0$) or subgingival plaque ($P = 1.0$).

Discussion

In the present study, 30 patients who tested urease positive in the stomach were evaluated by PCR in order to determine the presence of *H. pylori* in their mouth and stomach. All patients tested positive for *H. pylori* in the stomach and 43.3% of them tested positive in their mouth. This high prevalence of *H. pylori* in the mouth may be related to the poor oral hygiene of the patient population found in the study.

Table 1. Number of subjects positive for *H. pylori* in the gingivitis group

	Saliva	Plaque from dorsum of tongue	Supragingival plaque	Subgingival plaque
Positive	2	0	3	5
Negative	13	15	12	10

Table 2. Number of subjects positive for *H. pylori* in the chronic periodontitis group

	Saliva	Plaque from dorsum of tongue	Supragingival plaque	Subgingival plaque
Positive	1	0	3	3
Negative	14	15	12	12

Avcu et al. (3) observed that patients with poor oral hygiene were most likely to have *H. pylori* in their mouths. They also suggested that *H. pylori* could recur in the stomach of these patients after triple therapy more frequently than in patients with good oral hygiene.

The findings of Andersen et al. (1) justify the suggestion that dental plaque serves as a reservoir for this pathogen. They demonstrated the ability of *H. pylori* to coaggregate to *Fusobacterium nucleatum and Fusobacterium periodonticum*, which are early and late colonizers of the mouth (1). On the other hand, some oral bacterial species can inhibit growth of *H. pylori*. Okuda et al. (19) observed that *Streptococcus oralis, Streptococcus mitis, Streptococcus mutans, Streptococcus sobrinus, Actinomyces naeslundii, Prevotella intermedia, and Prevotella nigrescens* produce bacteriocin-like inhibitory proteins against *H. pylori*. The fact that good oral hygiene patients harbor less *H. pylori* in their mouths (3) could also be explained by this inhibitory activity of the early colonizers of the mouth. In addition, *P. intermedia* is not found in all gingivitis and periodontitis patients (7, 24). This could explain why some patients in our study who tested positive for *H. pylori* in the stomach and had poor oral hygiene, did not harbor the bacteria in their mouth.

The findings of studies evaluating the presence of *H. pylori* in the human mouth are equivocal, due to differences in study populations, sample collection, and in methods employed for detection. PCR is a more sensitive method of detection for *H. pylori* than other methods, detecting as few as 10 bacterial cells (6). However, the specificity and sensitivity of the method may vary. The primers used in this study (JW22 and JW 23) have been previously tested for their specificity and sensitivity. Those test results have shown both a high specificity, since only *H. pylori* DNA was amplified, and a high sensitivity, with a lower limit of detection of 100 fg of bacterial genomic DNA (22). The 30 patients evaluated in this study were urease positive and all biopsy specimens obtained in the endoscopic exam tested positive for *H. pylori*, validating the PCR method employed.

There are differing points of view in the literature concerning the contention that the oral cavity is a reservoir of *H. pylori*. Some authors believe that *H. pylori* have only a transient presence in the oral cavity (18). The antagonistic effects of some oral bacteria to *H. pylori*, demonstrated *in vitro*, could inhibit colonization by this organism in the oral cavity (11). Authors who detected low percentages of *H. pylori* in the mouths of their patients consider that it is not a significant microenvironment for this bacterium (2, 6, 21). On the other hand, authors who found this bacterium in almost all of their studied population consider it part of the normal microbiota of the oral cavity (25, 26). Our opinion is that the mouth should be considered an important reservoir of *H. pylori* since this bacterium can be found in dental plaque as well as in saliva, posing a threat for reinfection of the stomach. The work by Miyabayashi et al. (15) corroborates our findings. They reported a lower eradication rate for subjects harboring *H. pylori* in their mouths, and reinfection of the stomach in two of these patients who had had a successful eradication therapy.

Dental plaque may harbor *H. pylori*, as has been shown by many studies. It is important to note that supra- and subgingival plaque corresponds to different microenvironments when availability of nutrients, oxygen, and host defense mechanisms are considered (5, 27). Changes in these microenvironments may lead to different gingival clinical status (12). Although this may have an effect on the presence of this bacterium in dental plaque, few studies have separately investigated supra- and subgingival plaque, and most of them have not separated them at all (3, 6, 13, 21, 23, 25, 26). Two of the studies that investigated supra- and subgingival plaque separately did not evaluate the gingival status of their patients (17, 22). Our study evaluated supra- and subgingival plaque as well as gingival status. However, our results have shown that, despite the different characteristics of the evaluated sites, no statistically significant difference was observed between them regarding the prevalence of *H. pylori*. There was also no significant difference in the prevalence of this bacterium in gingivitis and chronic periodontitis patients.

The prevalence of *H. pylori* in subgingival plaque was investigated by the PCR method in different studies. Asikainen et al. (2) was not able to detect *H. pylori* in subgingival plaque of 336 patients with periodontitis, although no analysis for the presence of this bacterium in the stomach of those patients was performed in that study. On the other hand, Riggio & Lennon (22) evaluated patients that had no symptoms of gastritis or peptic ulcer disease. They observed *H. pylori* in the subgingival plaque of 38% of the adult periodontitis patients assessed. In the present study, the patient population exhibited *H. pylori* in the stomach. The bacterium was detected in subgingival plaque of eight patients out of 30 (26.6%). Three of these patients belonged to the chronic periodontitis group.

H. pylori have not been frequently detected on the dorsum of the tongue. Where there is a higher frequency, this has been associated with some type of tongue pathology. In our study, *H. pylori* were not detected on the dorsum of the tongue of any of the evaluated patients. Our results are in agreement with those of Oshowo et al. (20) who did not find this bacterium in any of 208 evaluated patients. Namavar et al. (16) observed the presence of *H. pylori* in only one sample from the tongue of one of 20 dyspeptic patients studied. On the other hand, Gall-Troselj et al. (10) detected *H. pylori* on the dorsum of the tongue of 43 (16%) of 268 evaluated samples. In their study, all patients had some type of tongue pathology such as atrophic glossitis, migratory glossitis, or burning mouth syndrome.

H. pylori were detected in the salivary samples from three patients (10%). Various prevalences in saliva have been reported in the literature. Oshowo et al. (20) did not find this bacterium in any of his samples.

Mapstone et al. (13) (23%) found a higher prevalence of *H. pylori* in saliva than found in our study. However, these results are quite different from Song et al.'s (26), who found the bacterium in 55% of salivary samples (26). The differences observed among the studies could be due to the diversity of the populations studied or to the specificity of the primers used.

The presence of *H. pylori* in the oral cavity may be a risk factor for recurrent gastric infection (15). Our results have shown that *H. pylori* can be found in different sites of the oral cavity of patients positive for these bacteria in the stomach. Thus, the possibility of reinfection of the stomach after treatment should not be discarded (15). More studies are needed to clarify whether these bacteria are eliminated from the oral sites as well as from the stomach after treatment.

The prevalence of *H. pylori* is similar for supra- and subgingival plaque. Periodontitis and gingivitis patients showed a high prevalence (43%) of *H. pylori* but there was no difference between these groups. Periodontitis and gingivitis patients may harbor *H. pylori* in saliva, supra- and subgingival plaque, and therefore constitutes a potential risk for reinfection of the stomach.

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