Helicobacter pylori detection in gastric biopsies, saliva and dental plaque of Brazilian dyspeptic patients

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Helicobacter pylori is an important human pathogen that causes chronic gastritis and is associated with the development of peptic ulcer disease and gastric malignancies. The oral cavity has been implicated as a potential H. pylori reservoir and may therefore be involved in the re-infection of the stomach, which can sometimes occur following treatment of an H. pylori infection. The objectives of this paper were (i) to determine the presence of H. pylori in the oral cavity and (ii) to examine the relationship between oral H. pylori and subsequent gastritis. Gastric biopsies, saliva samples and dental plaques were obtained from 78 dyspeptic adults. DNA was extracted and evaluated for the presence of H. pylori using polymerase chain reaction and Southern blotting methods. Persons with gastritis were frequently positive for H. pylori in their stomachs (p < 0.0001) and there was a statistically significant correlation between the presence of H. pylori in gastric biopsies and the oral cavity (p < 0.0001). Our results suggest a relationship between gastric infection and the presence of this bacterium in the oral cavity. Despite this, H. pylori were present in the oral cavity with variable distribution between saliva and dental plaques, suggesting the existence of a reservoir for the species and a potential association with gastric re-infection.

Key words: Helicobacter pylori - oral infection - saliva - dental plaque

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significantly greater risk of gastric reinfection following successful therapy. Thus, this study emphasised a clear link between the presence of *H. pylori* in the oral cavity and the infection of gastric mucosa (Loster et al. 2006).

Previous research has laid the groundwork for the present paper. *H. pylori* has been suggested to colonise the mouth of persons with gastritis; however, the support for this hypothesis is unclear. Therefore, the objectives of this paper were (i) to determine the presence of *H. pylori* in the oral cavity and (ii) to examine the relationship between the presence of the bacteria in the oral cavity and gastric mucosa.

**PATIENTS, MATERIALS AND METHODS**

Seventy-eight adults (36 male and 42 female with a mean age 51.2 years) presenting recurrent abdominal pain participated in the study. All subjects were recruited from Ambulatório de Endoscopia of Faculdade de Medicina de Marília, São Paulo, Brazil. All subjects signed an informed consent form that was approved by the local Ethical Committee.

Three biopsies were obtained from the gastric antrum of each patient. The first antrum specimen was used for the rapid urease test, the second specimen was used for histology and the third for molecular analysis. Dental plaque and saliva was also acquired from each subject. Subjects who were HIV-positive or had taken anti-inflammatory or antimicrobials drugs within the previous two months were excluded from the study.

**Rapid urease test and histology** - Infection was determined by a Rapid urease test, using the TUPF kit (Laborclin, Brazil), according to the manufacturer’s instructions. Samples were examined within 24 h of collection. Urea hydrolysis and histopathological examinations were conducted to determine the presence of *H. pylori* in accordance with the updated Sydney System.

**Collection and DNA extraction from saliva and dental plaque** - Saliva and plaque samples were collected from each subject prior to endoscopic examination. Salivary flow was stimulated for each patient and 3 mL of saliva was collected in test tubes. Dental plaque from all regions of the oral cavity (incisors, canines, premolars and molars) was removed with a sterile curette and transferred to 15 mL of phosphate buffered saline.

DNA extraction from dental plaques and saliva was performed as previously described (Okada et al. 2000, Kigel et al. 2005). Briefly, 10 mL of dental plaque suspension or 1 mL saliva were centrifuged for 5 min at 10,000 rpm. The pellets were suspended in 480 μL of digestion buffer (5 mM EDTA, pH 8, 0.5 mol Tris-HCl, pH 7.5 and 5% Tween 20) and 20 μL of 100 μg/mL proteinase K and incubated at 55°C overnight. DNA was extracted twice with an equal volume of phenol-chloroform and then precipitated with a double volume of 100% ethanol. Finally, the extracted DNA was resuspended in 80 μL to 100 μL of TE buffer.

**Preparation of the DNA probe** - A 150-base-pair fragment was amplified by PCR from genomic DNA of cultured *H. pylori* using the Hpx1 and Hpx2 primers. This fragment was then used as the probe for the hybridisations. After amplification, the reaction mixture was electrophoresed in 2% agarose gels and the 150 base pair fragment of interest was recovered from an agarose gel and purified using GFX PCR DNA and Gel Band Purification Kit (Amersham Pharmacia, USA). The probe was synthesised using the Kit Gene Images AlkPhos Direct Labelling (Amersham Pharmacia, USA), according to the manufacturer’s instructions.

**Statistical analysis** - Statistical analysis was performed by χ² and Kappa test. The significance level was set at a p value of < 0.05.

**RESULTS**

Detection of *H. pylori* from gastric biopsies by PCR, Southern blotting, histology and urease test - We utilised several methods to test for the presence of *H. pylori* in gastric biopsies of all 78 adult patients, the results of which are presented in Table I. In our PCR analysis, 46 (59%) of the patients presented with *H. pylori* infection. Combining that data with the results of our Southern blots caused the number of *H. pylori* infected patients to increase from 46-66 (84.6%), with 25.6% of positive samples showing a significant increase (p < 0.0001) (Figure). In addition, we examined the histology of these biopsies and found that 54 (69.2%) patients had chronic gastritis and 24 (30.8%) patients demonstrated normal mucosa without gastric alterations. Furthermore, our histological analysis showed the presence of *H. pylori* in

|                  | Urease test n (%) | Histology n (%) | PCR n (%) | Southern blotting n (%) |
|------------------|------------------|----------------|----------|-------------------------|
| *H. pylori*-positive | 30 (38.5)       | 21 (27)        | 46 (59)  | 66* (84.6)              |
| *H. pylori*-negative | 48 (61.5)       | 57 (73)        | 32 (41)  | 12 (15.4)               |

Total 78 (100)

a: p < 0.0001.
21 (27%) subjects and the urease test detected *H. pylori* infection in 30 (38.5%) patients. It should be noted that both of these techniques showed decreased sensitivity and specificity when compared with the results of PCR and Southern blot hybridisation.

**Detection of *H. pylori* in saliva and dental plaque by Southern blotting** - We performed Southern blots of DNA extracts from the saliva and dental plaque of all 78 patients. *H. pylori* was found in the saliva of 33 (42.3%) patients and in the dental plaque of 37 (47.4%) patients (Table II) (Figure). Of the 66 patients who were *H. pylori* positive in their gastric biopsies, 19 (28.8%) were found not to have *H. pylori* in the oral cavity. Of the 12 patients whose gastric biopsies were negative for *H. pylori*, six (50%) were found not to have *H. pylori* in the oral cavity. In total, *H. pylori* DNA was detected in all three samples - gastric mucosa, saliva and dental plaque - in 14 (21%) patients. *H. pylori* was detected in gastric biopsies and either the saliva or the dental plaque in 33 (50%) subjects.

No statistically significant difference was observed between strains in the saliva and dental plaque. However, a statistically significant correlation was observed between the presence of *H. pylori* in the gastric biopsies and the oral cavity (p < 0.0001).

**DISCUSSION**

*H. pylori* is the causative agent of chronic superficial gastritis and plays an important role in the aetiology of peptic ulcer disease. Evidence suggests that *H. pylori* infection pre-exists the development of gastric carcinoma and is a risk factor for the development of other gastric diseases (Gatti et al. 2005). Because of the importance of this bacterium in the development of chronic gastritis, we evaluated the association between the presence of *H. pylori* in gastric biopsies and in the saliva and dental plaques of the same dyspeptic adult individuals.

Previous studies (Tiwari et al. 2005, Loster et al. 2006, Souto & Colombo 2008, Harris et al. 2008) used PCR to diagnose an infection of *H. pylori*. These results are comparable to the results of the present study, in which there was a 95% success rate in the detection of *H. pylori* in the stomach. Li et al. (1995) and Song et al. (2000) showed a significant increase in the sensitivity of detection after using Southern blotting and also verified a high prevalence of infection.

The high sensitivity and specificity of the PCR test with hybridisation and the probe used and synthesised from genomic DNA of cultured *H. pylori* decrease the possibility of finding false positives or false negatives or of contamination (Clayton et al. 1991, Song et al. 1999). In addition, we used a pair of primers, designated Hpx1/ Hpx2, that were specific for a 150 bp fragment of 16S rRNA of *H. pylori*, which is the most conserved region of the genome, introducing a higher sensitivity and specificity when compared to other primers specific to *H. pylori*.

Our results parallel previous studies and have revealed that the prevalence of the organism in adults can exceed 80%. However, it is important to remember that the infection rates can vary dramatically by geographic area, age, race and socioeconomic status (Mitchell et al. 2003, Souto & Colombo 2008).

**TABLE II**

|                      | Gastric mucosa n (%) | Saliva n (%) | Dental plaque n (%) |
|----------------------|----------------------|--------------|---------------------|
| *H. pylori*-positive | 66* (84.6)           | 33 (42.3)    | 37 (47.4)           |
| *H. pylori*-negative | 12 (15.4)            | 45 (57.7)    | 41 (52.6)           |

Total 78 (100)

a: p < 0.0001.

A: agarose gel 2% showing *Helicobacter pylori* PCR products of strains of gastric biopsies, saliva and dental plaques (Slots 1, 3, 5, 7: saliva samples; 2, 4, 6, 8: dental plaques samples; 9-12: gastric biopsies samples; 13, 15: controls positive and negative, respectively; 14: Ladder Marker 100 bp (Invitrogen); B: autoradiograph after hybridization with specific chemiluminescent probe referent to A (Slots 1, 5, 7: *H. pylori*-negative saliva samples; 3: *H. pylori*-positive saliva sample; 2, 4, 6, 8: *H. pylori*-positive dental plaques samples; 10: *H. pylori*-negative gastric biopsy sample; 9, 11, 12: *H. pylori*-positive gastric biopsies samples; 13, 15: control positive and negative, respectively).
Several authors (Madmujar et al. 1990, Krajden et al. 1989, Nguyen et al. 1995, Loster et al. 2006, De Sousa et al. 2006, Souto & Colombo 2008, Silva et al. 2009) have reported that the oral cavity can be a reservoir for H. pylori, making treatment difficult and exposing the individual to a higher risk of gastric reinfection. However, Okada et al. (2000), Dye et al. (2002) and Olivier et al. (2006) have characterised the bacterium in the oral cavity as being mainly transient. Despite this, these authors do not discard the hypothesis that an association between the presence of the bacterium in the stomach and mouth may exist.

In our study, 33 (42.3%) and 37 (47.4%) of the patients had H. pylori in saliva and dental plaque samples, respectively. Li et al. (1995) found 75% of saliva samples to be positive, which is similar to the results of Wang et al. (2002), who found the bacterium in 71% of saliva samples. Tiwari et al. (2005) studied 120 dyspeptic patients and detected H. pylori in the gastric and saliva samples from each patient. Both authors suggested that saliva may be a method of transmission and may potentially induce gastric reinfections, which supports the results of the present study.

Song et al. (2000) analysed 117 samples of dental plaque from 42 patients and verified the presence of the bacterium in 68% of the samples. These results are similar to those of Liu et al. (2009), who found positive samples in 59% of their subjects. It is interesting to note that these studies differ from the results of Kigeln et al. (2005), who found a low prevalence in gastric biopsy samples and only one positive dental plaque sample. Similarly, Souto and Colombo (2008) found that 20% and 33% of subjects had positive samples in saliva and dental plaque, respectively.

Silva et al. (2009) used a control group (individuals with no gastric disease who were H. pylori positive) and a case group (individuals with gastric disease who were H. pylori positive). The results from this study showed the presence of H. pylori in saliva and dental plaques from only the patients in the case group, suggesting an association between the oral cavity and gastric disease.

Inconsistent differences in the frequency of H. pylori in the oral cavity are found in the literature. These differences may be a consequence of variations in the demographics of subjects, oral health status, H. pylori infection status, type and number of clinical samples, complexity of the oral microbiota and methods of detection (Souto & Colombo 2008).

Kigeln et al. (2005) reported that the levels of bacteria in the oral cavity may be too low to be detected by one round of PCR. They further emphasised that the location in the mouth used for the collection of the samples can influence the prevalence of the microorganism. This idea was further supported by the results of Song et al. (2000), who found a prevalence of 82% in the molar region, 64% in pre-molar region and 59% in the incisor region. Loster et al. (2006) suggested that this variation may be the result of dental plaques being exposed to different levels of oxygenation, which can affect colonisation by H. pylori. The prevalence of infection in saliva and dental plaques, collectively, was 70% in this study.

In sum, our results verified a possible correlation between the prevalence of H. pylori in the oral cavity and infection in the stomach in Brazilian adult patients.

Our results suggest a relationship between gastric infection and the presence of H. pylori in the oral cavity. H. pylori were present in the oral cavity with a variable distribution between saliva and dental plaques, suggesting the existence of a reservoir for the species and a potential association with gastric reinfection.

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