The human stomach is considered the primary reservoir of *Helicobacter pylori* (*H. pylori*). Although the exact mode of transmission of *H. pylori* is uncertain, success in isolating *H. pylori* from dental plaque (DP) of patients in 1989 drew attention to the possible importance of oral-oral transmission.\(^1\) Accumulated data have shown a significant correlation between *H. pylori* infection of the mouth and the stomach.\(^2-4\) DP provides an optimal pH, temperature and microaerophilic environment required for the survival of *H. pylori*.\(^5\) Occurrence of the same strain of *H. pylori* in the stomach and DP has been reported.\(^2-4\) A study from India showed that systemic therapy failed to clear *H. pylori* from DP, despite its clearance from the stomach.\(^6\) These observations suggest that DP may be a potential source for the transmission of *H. pylori* and may possibly serve as a reservoir for reinfection.

**Background.** Den tal plaque has been suggested as a permanent reservoir of *Helicobacter pylori* (*H. pylori*) and a potential source of reinfection. The aims of this study were to investigate the presence of *H. pylori* in both dental plaque and the stomach and to evaluate the therapeutic effect of triple therapy on *H. pylori* in dental plaque.

**Methods.** Den tal plaque and gastric biopsy samples were obtained from 65 patients with dyspeptic symptoms for endoscopic examination. The prevalence of *H. pylori* in dental plaque and stomach was determined with rapid urease test, histologic examinations and polymerase chain reaction as say based on the primer pair derived from the *cagA* gene of *H. pylori*. Triple therapy was administered to patients infected with *H. pylori*. *H. pylori* status was re-evaluated after eradication therapy.

**Results.** Prior to treatment, *H. pylori* was found in the stomach in 38 of 65 (58%) patients and in dental plaque in 28 of 65 (43%) patients. The coexisting infection rate of *H. pylori* in both stomach and dental plaque was 74%. After triple therapy, *H. pylori* was eradicated from the stomach in 32 of 38 (84%) patients, but only 2 of 28 (7%) patients with coexisting *H. pylori* infection of stomach and dental plaque showed the elimination of *H. pylori* from dental plaque.

**Conclusions.** The high coexisting infection rate of *H. pylori* in both stomach and dental plaque implies that dental plaque can serve as another reservoir of *H. pylori*. *H. pylori* in dental plaque was hardly eradicated by triple therapy. Den tal plaque may be a potential source for recurrent episodes of gastric infection after successful systemic therapy.

**Key Words**

dental plaque; *Helicobacter pylori*; polymerase chain reaction

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or re crucence of infection after eradication treatment.

The aims of this study were to investigate the co-existing infection rate of *H. pylori* in both DP and stomach and to evaluate the therapeutic effect of triple therapy on *H. pylori* in DP.

**Methods**

**Patients**

Sixty-five consecutive patients with dyspeptic symptoms referred to our division for endoscopic examination were included in this study. Patients who had been taking H2-receptor antagonists, bismuth compounds, proton pump inhibitors, or antibiotics in the preceding 4 weeks were excluded. Our series was made up of 44 men and 21 women with a mean age of 48 years (range 18-67 years). Informed consent was obtained from all patients.

Patients were instructed to avoid oral hygiene in the morning of the day of examination. Before endoscopy, two samples of DP were collected from subgingival or supragingival plaque with a sterile curette. One of the DP samples was sent for rapid urease test, and the other was sent for polymerase chain reaction (PCR) assay.

Endoscopy was performed with an Olympus® GIF-XQ230 gastroduodenoscope. Biopsy forceps were sterilized and endoscopes were fully disinfected using an automatic washer (Olympus® Endoscope Washer EW-30, Tokyo, Japan) before and after each examination. During endoscopy, six biopsies were taken from the gas tric antrum and corpus. Two gastric samples, one from the antrum and the other from the corpus, were subjected to rapid urease test. An other two gastric specimens were sent for histological examination, and the remaining specimens were for PCR assay.

**Rapid urease test**

Samples from DP and the stomach for rapid urease test were immediately inoculated into the rapid urease test gel (CLO test, Tri-Med Specialties, Western Australia) after collection, and readings were taken for up to 24 hours.

**Histologic examination**

Two gastric biopsy specimens for histological examination were set in formalin, processed routinely, embedded in paraffin wax, and stained with hematoxylin and eosin to show bacteria histologically.

**PCR assay**

Gastric samples for PCR were immediately frozen in liquid nitrogen and digested overnight in a lysis buffer containing 100 µg/ml proteinase K, 10 mM Tris-HCl (pH 8.0), EDTA (pH 8.0) 0.1 M, and 0.5% SDS, then subjected to phenol/chloroform extraction. After alcohol precipitation, the DNA pellet was dissolved in 100 µL of water. The dental dust was boiled in 100 µL of lysis buffer for 15 min. After centrifugation, the supernatant was collected and further purified by phenol-chloroform. The precipitate was discarded.

Ten microliters of each DNA solution was subjected to a two-step nested PCR using two primer pairs from the cytotoxic associate gene A (cagA) of the *H. pylori* genome. PCR was carried out in a volume of 25 µL containing 50 mM potassium chloride, 10 mM Tris-HCl (pH 8.3), 1.5 mM magnesium chloride, 0.01% (W/V) gelatin, 250 µM of each of four dNTPs (Pharmacia), 0.6 µM of each primer, and 1.25 units of recombinant Taq DNA polymerase (Perkin-Elmer Cetus, USA). The sequence of the oligonucleotide, the conditions for PCR analysis, and the expected size of the PCR product are listed in Table 1. The amplification reaction using a thermal cycler (Perkin Elmer Cetus) consisted of 95 °C for 5 min for initial denaturation when using genomic DNA, followed by 35 cycles of denaturation at 95 °C for 20 s, (annealing reaction is shown in Table 1), and extension at 72°C for 30 s. Reagents with out DNA were used as negative controls in the PCR as say. For each round of PCR, cloned DNA fragments in TA-type cloning vector were used as a positive control to avoid
false-negative reactions due to the presence of Taq inhibitory substrates in the samples. Ten microliters of PCR product was electrophoresed on 1.5% agarose gel, then stained with ethidium bromide and photographed under ultraviolet light illumination.

**Treatment protocol**

If gastric samples of patients were positive by rapid urease test and bacteria were observed in the gastric mucosa on hematoxylin- and eosin-stained sections, those patients received bismuth-based classical triple therapy (colloidal bismuth subcitrate 1 gm plus amoxicillin 500 mg plus metronidazole 250 mg four times daily for 2 weeks) or cimetidine-based triple therapy (cimetidine 200 mg plus amoxicillin 500 mg and metronidazole 250 mg four times a day for 2 weeks). If gastric and/or duodenal ulceration was detected on endoscopic examination, cimetidine 400 mg twice a day was given for an additional 4 weeks.

One month after eradication therapy, DP sampling and endoscopic gastric biopsy were repeated to determine the *H. pylori* status. Eradication of *H. pylori* from DP was defined as the sample from DP being negative for PCR test. *H. pylori* from the stomach was considered eradicated if samples from the stomach were negative for both urease test and PCR test and if no bacteria were identified on hematoxylin- and eosin-stained sections.

**Statistical analysis**

The statistical difference between the eradication rates of *H. pylori* from the stomach and DP was analyzed using the Chi-Square test, with *p* values less than 0.05 being regarded as statistically significant.

**Results**

The PCR amplification of *cag A* of *H. pylori* is shown in Fig. 1. Prior to treatment, 38 out of 65 patients (58%) were *H. pylori*-positive in gastric samples. Twenty-eight patients had a positive PCR test in DP samples, and all of these patients had *H. pylori* infection in the stomach (Table 2). The prevalence rate of *H. pylori* in DP was 43%, and the coexisting infection rate of DP and stomach was 74%. All samples of DP were positive for rapid urease test, even in the absence of *H. pylori* in the stomach.

![Fig. 1.](image)

**Table 1. Sequence of oligonucleotides and conditions for polymerase chain reaction (PCR) analysis**

<table>
<thead>
<tr>
<th>Forward primers</th>
<th>Reverse primers</th>
<th>PCR conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'GAATTCAAAAATGGCA 3'</td>
<td>O6 5'ATACCGCTTGATTGAGATTG 3'</td>
<td>51°C 40 sec 35 cycle 685 bases</td>
</tr>
<tr>
<td>5'AATAAGGATTTGCAAAGGC 3'</td>
<td>I6 5'CAATTCTTGATTCTTGAAG 3'</td>
<td>47°C 90 sec 40 cycle 634 bases</td>
</tr>
</tbody>
</table>
Table 2. The eradication rates of \textit{H. pylori} from the stomach and from the dental plaque after triple therapy

<table>
<thead>
<tr>
<th>\textit{H. pylori}</th>
<th>Before treatment No. of patients (%)</th>
<th>After treatment No. of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stomach</td>
<td>Dental plaque</td>
</tr>
<tr>
<td>Positive</td>
<td>38/65 (58)</td>
<td>28/65 (43)</td>
</tr>
<tr>
<td>Negative</td>
<td>27/65 (42)</td>
<td>37/65 (57)</td>
</tr>
</tbody>
</table>

\(a\) \(p < 0.005\).

Of the 38 patients infected with \textit{H. pylori}, 32 patients had cleared \textit{H. pylori} from the stomach after triple therapy. The eradication rate of \textit{H. pylori} from the stomach was 84\%. However, among the 28 patients who had \textit{H. pylori} infection in both stomach and DP, only two patients cleared \textit{H. pylori} from DP, and the eradication rate of \textit{H. pylori} from DP was 7\%. There was statistically significant difference between the eradication rate of \textit{H. pylori} from the stomach and from DP (\(p < 0.005\)).

**Discussion**

\textit{H. pylori} has been identified in DP by many studies. Using different methods, the infection rate of \textit{H. pylori} in DP ranges from 0\% to 100\%. By rapid urease test, \textit{H. pylori} was identified in almost 100\% of cases. However, a positive urease test on a sample obtained from DP should be interpreted with caution. Some \textit{H. pylori}-like organisms which are urease-, catalase-, and oxidase-positive have been isolated from DP. Other urease-positive bacteria, for instance, \textit{Actinomyces} species, \textit{Streptococcus} species, \textit{Micrococcus} species, \textit{Haemophilus} species, \textit{Bacteroides ureolyticus}, and \textit{Stomatococcus mucilaginosus}, are commonly present in DP, which may cause false-positive results of the urease test.

In our results, all specimens from DP of our patients were positive for urease tests, even when there was no evidence of the presence of \textit{H. pylori} in the stomach. Therefore, we suggest that it is incorrect to use the urease test as an indicator of the presence of \textit{H. pylori} in DP. Contrarily, present cultural methods are inadequate for reliably isolating \textit{H. pylori} from DP. The reasons for low reproducibility rates of \textit{H. pylori} from DP include the uneven distribution of scanty \textit{H. pylori} in DP causing inadequacy in sample collection and the presence of nonculturable coccoid forms of \textit{H. pylori} in the mouth. More over, DP harbors many other bacterial species, mostly Gram-positive and anaerobic fusiforms, and their concomitant presence may have an inhibiting effect on isolation of \textit{H. pylori} from DP.

PCR as a rapid diagnostic test for \textit{H. pylori} in samples taken from sites outside the stomach has provided the most sensitive and specific test for the detection of \textit{H. pylori} in previous series. Because urease-positive organisms are commonly present in the mouth, reports of high detection rates of \textit{H. pylori} in the oral cavity based on primer pairs derived from the urease gene have been questioned.

Fig. 2. Diagram presenting plaque bacteria and \textit{H. pylori} as so ciated with tooth surface and periodontal tissue pocket.
have the \textit{cagA} gene.\textsuperscript{20} We designed a highly specific probe derived from the \textit{cagA} gene of \textit{H. pylori}.\textsuperscript{7} Based on the sequence of this probe, \textit{H. pylori} was positive in DP of 48% patients in our study. In addition, this study also shows that among the 38 patients infected with \textit{H. pylori}, the existing infection rate of DP and stomach was 74%. DP therefore could be implicated as an important reservoir of \textit{H. pylori}.

After triple therapy, \textit{H. pylori} was eradicated from the stomach in 84% of patients, but only 7% of patients were shown to be clear of \textit{H. pylori} from DP. Low eradication rates of \textit{H. pylori} from DP have been reported by previous studies.\textsuperscript{5,8} The lack of effect of systemic therapy on \textit{H. pylori} in DP may be ex plained by the structure of DP (Fig. 2). There are several different zones in the bottom of a periodontal pocket, namely the calculus, attached plaque, unattached plaque, and junctional epithelium.\textsuperscript{21} DP consists primarily of proliferating microorganisms, along with a scattering of epithelial cells, leukocytes, and macrophages in an adherent intracellular matrix.\textsuperscript{22} Calculus represents mineralized bacterial plaque, and it contains usually harbors viable bacteria.\textsuperscript{21} \textit{H. pylori} harbored in DP or calculus may be inaccessible to systemic antibiotic therapy.\textsuperscript{10}

According to long-term follow-up studies, the reinfection rate of \textit{H. pylori} after successful systemic therapy is low and approximately 1% per year in both developed and developing countries;\textsuperscript{23,24} however, re infection of the stomach by the identical strain has been reported.\textsuperscript{25} Our study shows that triple therapy cleared \textit{H. pylori} from stomach in 84% of patients, but only in 7% of patients from DP. Accordingly, we conclude that DP serves as a permanent reservoir of \textit{H. pylori}, and it also is a potential source for gastric re infection after successful systemic therapy. Local treatment or other therapeutic modalities might be needed to eradicate \textit{H. pylori} from DP.

References