DETECTION OF HELICOBACTER PYLORI
COLONIZATION IN HUMAN DENTAL PLAQUES AND
SALIVA OF PATIENTS WITH CHRONIC GASTRITIS

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ABSTRACT

Recently, dental plaque (DP) and saliva have been implicated as possible sources of Helicobacter pylori infection. This subject was studied to investigate the detection rates of H. pylori in DP and saliva by use of EIA, CLO tests and culture depending on H. pylori infection state of gastric mucosa. H. pylori positive was found in 68.3% (468) by CLO test, 46.4% (318) by EIA and 43% (295) by culture in gastric mucosa samples taken from 685 patients. CLO positive of 468 patients in dental plaque is found from dentate patients 11.1%, edentulous patients 16.23% and saliva from dentate patients 2.77%, edentulous patients 4.05%. H. pylori positive by EIA 318 patients’ in dental plaque is found from dentate patients 9.1%, edentulous patients 14.46% and saliva from dentate patients 1.57%, edentulous patients 0.33%. H. pylori positive by culture 295 patients’ in dental plaque is identified from dentate patients by culture 5.42%, edentulous patients 9.15% and saliva from dentate patients 0%, edentulous patients 0.33%. The detection rates of H. pylori in DP (6.9%) were rather low than saliva (28.6%) respectively. About half of the world population is infected with H. pylori, but the transmission and the source of this infection are still unknown.

Introduction

Helicobacter pylori is a microaerophilic, spiral-shaped, motile, gram-negative bacterium and is strongly associated with gastrointestinal disease, including chronic active gastritis, peptic and duodenal ulcer disease, and gastric cancer (1-2). H. pylori infection is widespread throughout the world, and about half of the world population is infected with H. pylori (3). The mode of transmission, the natural history, and other aspects of the epidemiology of H. pylori infection are still unclear. Reported observations support a person-to-person mode of transmission via fecal-oral, oral-oral, and gastro-oral routes (4, 5).

Recently, dental plaque (DP) and saliva have been implicated as possible sources of H. pylori infection (6). To establish the prevalence of H. pylori colonization of DP and its correlation with Helicobacter pylori infection of the antral mucosa in patients with symptomatic dyspepsia (3). DP may be a reservoir for H. pylori, which is probably transmitted by person-to-person contact. Community education about effective oral hygiene and adoption of good hygiene practices by those with regular public contact may be important to prevent acquisition and transmission of H. pylori (1, 7). It has long been speculated that DP might harbour H. pylori and therefore might be a source of reinfection of the gastric mucosa. Some authors have suggested that H. pylori may belong to the normal oral flora of the human oral cavity, maintaining a commensal relationship with the host, but present in very low numbers such that reliable detection is difficult (8).

Several methods for detecting H. pylori
are used at present. Most of these diagnostic tests are performed on gastric biopsy samples, and the bacterium can be identified in these specimens by urea hydrolysis test, staining techniques, and culturing (6, 9). The possibility that *H. pylori* may colonize the oral cavity has attracted considerable attention (10). The presence of *H. pylori* in dental plaque of patients both with and without stomach disorders has been investigated by bacterial culture, CLO and EIA methods.

Most studies have failed to isolate *H. pylori* by culture from dental plaque of subjects with gastric infection (10, 11, 12). In this study, our aim was to establish the prevalence of *H. pylori* in dental plaque and saliva of patients with chronic gastritis.

**Materials and Methods**

685 patients (321 men, 364 women) who have the complaint of chronic gastritis randomly chosen, were included in this study. Gastric mucus from gastric mucosa of antrum and body of stomach were collected from all 685 participants with soft gastroscopic brush. Oral specimens, consisting of dental plaque were collected from dentate patients with chronic gastritis using dental scaling hand instruments. Denture plaque was obtained from the fitting surfaces of dentures from edentulous patients with chronic gastritis (13). Saliva, were collected with sterile toothpicks and filter paper, respectively, from 685 patients with endoscopically diagnosed gastroduodenal ulcer disease attending the Gastroenterology Unit at State Hospital, Diyarbakir, Turkey. *H. pylori* colonization was evaluated with CLO test, microscopy of Gram stained mucosal smear, culture and histology after modified Giemsa staining in the antrum and body, respectively. All specimens were dispersed in modified urea broth and normal saline solution before being inoculated onto selective Skirrow's agar and onto brain heart infusion agar plates (Merck, Darmstadt, Germany) supplemented with 5% (vol/vol) sheep blood and incubated in a microaerophilic atmosphere.

Plasma samples were tested for *H. pylori* IgG antibodies with the Pyloriset EIA-G Kit (Pyloriset, Orion Corporation, Orion Diagnostica, Espoo, Finland). A cut-off antibody titre of 500 was used to classify subjects as positive or negative, as recommended by the manufacturer (sensitivity, 92.5%; specificity, 84.3%).

Histologic confirmation of *H. pylori* status: Gastric mucosal biopsy specimens were taken with jumbo forceps (13-K with spike removed; Olympus, Lake Success, N.Y.) Biopsy specimens, which were fixed in 10% buffered formalin, were processed, oriented on edge, embedded in paraffin, and cut in sequential 4 m sections. Virtually all specimens included surface epithelium and muscularis mucosa. The specimens were stained with hematoxylin and eosin. Samples of dental plaque were air-dried and stained with Giemsa stain. *H. pylori* appears as dark bluish violet, curved, spiral-shaped rods approximately 2-3 m long (14).

A comparative evaluation of saliva, dental plaque and gastric mucosa urease testing (Complobacter-Like Organism Test – CLO-, Delta West (USA) histologic research, serology (IgG Anti- *Helicobacter pylori* using enzyme linked immunosorbent assay) and culture were done.

**Results and Discussion**

Sixhundredeightyfive patients (321 men, 364 women) were included in this study. *Helicobacter pylori* was detected by the CLO test, EIA and culture in gastric mucosa. The results obtained in gastric mucosa are shown in Table 1. *H. pylori* could be detected in the gastric mucosa by CLO tests in 468 of 685 patients (68.3%); by EIA tests in 318 of 685 patients (46.6%), and 295 patients showed positive *H. pylori* culture in gastric mucus (43%) (Table 1). *H. pylori* was identified in dental plaque
TABLE 1
Presence of *H. pylori* in dental plaque of patients both dentate and edentulous patients investigated by CLO test method, EIA and bacterial culture

<table>
<thead>
<tr>
<th>n:685</th>
<th>CLO TEST Positive</th>
<th>EIA Positive</th>
<th>CULTURE Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric mucosa Patients</td>
<td>%</td>
<td>Patients</td>
<td>%</td>
</tr>
<tr>
<td>Dentate</td>
<td>468</td>
<td>68.3</td>
<td>318</td>
</tr>
<tr>
<td>Edentulous</td>
<td>468</td>
<td>68.3</td>
<td>318</td>
</tr>
</tbody>
</table>

**TABLE 2**
Helicobacter pylori gastric mucosa positive (CLO) patients

<table>
<thead>
<tr>
<th>N=468</th>
<th>Patients</th>
<th>Number %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dental plaque</td>
<td>Dentate</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Edentulous</td>
<td>66</td>
</tr>
<tr>
<td>Saliva</td>
<td>Dentate</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Edentulous</td>
<td>19</td>
</tr>
</tbody>
</table>

**TABLE 3**
Helicobacter pylori gastric mucosa positive (EIA) patients

<table>
<thead>
<tr>
<th>N=318</th>
<th>Patients</th>
<th>Number %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dental plaque</td>
<td>Dentate</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Edentulous</td>
<td>46</td>
</tr>
<tr>
<td>Saliva</td>
<td>Dentate</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Edentulous</td>
<td>11</td>
</tr>
</tbody>
</table>

samples by CLO test in 52 dentate patients (11.11%), denture plaque samples from 76 edentulous patients (16.23%), in saliva samples from 13 dentate patients (2.77%) and from 19 edentulous patients (4.05%) (Table 2) (Fig. 1).

EIA test allowed to identify *H. pylori* in dental plaque samples from 29 dentate patients (9.11%), denture plaque samples from 46 edentulous patients (14.46%), and saliva samples from 5 dentate patients (1.57%), and from 11 edentulous patients (3.46%) (Table 3) (Fig. 2)

*H. pylori* cultures were positive in dental plaque samples from 16 dentate patients (5.42%), denture plaque samples from 27 edentulous patients (9.15%), and from only 1 edentulous patients (0.33%) (Table 4) (Fig. 3).

In adults, previously investigated risk factors for *H. pylori* include race-ethnicity, socioeconomic status, education, dental prosthesis and irregular dental treatment, public contact, working with animals, smoking status, alcohol consumption, homosexuality, sexually transmitted diseases, and having an infected child or partner (7, 15). It is uncertain from where and how *H. pylori* is acquired, but most investigators seem to favour person-to-person transmission (16). The prevalence of *H. pylori* in dental plaque of patients with dyspepsia was very high in our patients indicating it to be a major reservoir of infection (2). Nevertheless, the considerable proportions of gastroduodenal ulcers, peptic ulcer bleeds and gastric cancers attributable to *H. pylori* make an infection rate of 30.6% an
**TABLE 4**

<table>
<thead>
<tr>
<th>Helicobacter pylori gastric mucosa positive (Culture) patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=295 Patients Number %</td>
</tr>
<tr>
<td>Dentate</td>
</tr>
<tr>
<td>Edentulous</td>
</tr>
<tr>
<td>Saliva</td>
</tr>
</tbody>
</table>

**Helicobacter pylori** gastric mucosa positive (Culture) patients

Fig. 3. *Helicobacter pylori* was detected by bacterial culture in saliva and dental plaque.

Important public health issue (17).

Eradication of *H pylori* from gastric mucosa alone is not enough to prevent gastric recurrence of the bacteria. Proper oral hygiene must be established to eliminate *H pylori* in dental plaque. Therefore, we suggest that control of *H. pylori* in dental plaque is necessary to control recurrence of *H. pylori* (11).

In the present study, our aim was to determine the prevalence of *H. pylori* colonization in dental plaque and saliva of patients with chronic gastritis in Turkey.

The rate of *H. pylori* acquisition is higher in developing countries than in developed countries. It is also very high in Turkey, and these high incidences of *H. pylori* may be related to cultural background, diet, or environmental factors. Another main factor may be poor oral hygiene that leads the formation of dental plaque. The area around dental plaque has a low reduction-oxidation reaction potential, which promotes growth of facultative anaerobes (11).

Biofilm, like dental plaque, is a ready source for reinfection. They are complex communities of many bacterial species with powerful defences against chemical and pharmacological threats, but some organisms may not gain a foothold in them because of bacterial antagonisms (10). This means that not all patients with *H. pylori* infection will necessarily have the organism in their dental plaque, which may have misled some investigators in the past. Reinfection from plaque will also be subject to variable host defences and therefore not occur consistently in all cases in which conventional *H. pylori* treatment is used.

Our results are comparable with most reports from other parts of the world. Despite the potential problems with culture of the *H. pylori* from dental plaque samples, there have been two reports of its isolation from dental plaque. The frequency of isolation has varied; Krajden et al. (18) in Canada reported that for only 1 of 29 patients (3.4%) with *H. pylori* gastritis was *H. pylori* recoverable from their dental plaques. In contrast, in India *H. pylori* was present in 100% of 40 volunteers (19).

In a study conducted in India, with the CLO test, *H. pylori* was detected in dental plaque and in gastric antral and body mucosa in 98%, 67% and 70%, respectively, of 43 consecutive patients with dyspepsia. The rapidity of the CLO test indicates that the density of *H. pylori* was the highest in dental plaque, less in the antrum, and least in the body mucosa of the stomach (20).

In the study of Özdemir et al., chronic gastritis was diagnosed in 63 (77.7%) of 81 patients. Dental plaque samples of 64 (79%) patients and tongue scraping samples of 48 (59.2%) patients were urease positive. Of the 63 patients with chronic gastritis, dental plaque and tongue scarrings were urease positive in 52 (83%) and 37 (59%) patients, respectively (21).

In research of Butt et al. found that by CLO test 44 out of 69 patients (63%) were
both dental plaque and antral biopsy positive for *H. pylori*. No patient with negative dental plaque cytology was positive for *H. pylori* in gastric mucosa. A statistically significant correlation was found between *H. pylori* colonization of dental plaque and gastric antrum (3).

Dental scaling hand instruments were used to collect supragingival and subgingival dental plaque from 81 dentate participants. Denture plaque was obtained from the fitting surfaces of dentures from 41 edentulous patients. Dental plaque from all dentate participants was negative for *H. pylori* culture. Only one 80-year-old edentulous patient had positive *H. pylori* culture in both gastric mucus and denture plaque (13).

Cammarota et al. found that *H. pylori* had a low prevalence (3.2%) in the oral cavity, with no significant relationship between gastric mucosa and dental plaque colonization (22).

Kamat et al. obtained dental plaque from 156 patients and 92 healthy volunteers were also evaluated for the presence of *H. pylori* using rapid urease test (RUT) and culture (23).

In research done in Pakistan, *H. pylori* dental plaque colonization in 125 males and 53 females (group I) attending a dental clinic in Pakistan. CLO test was positive in all specimens, while cytology for *H. pylori* using rapid urease test (RUT) and culture (22).

In another research done in Belgium, including 603 staff members, and 439 in the control group *H. pylori* antibodies prevalence has been researched. *H. pylori* antibodies in staff members was recorded as 40.6%, and as 29.2% in the control group (27). The *H. pylori* seropositivity rate was higher in Japanese (72%), than in Dutch (33%) (22). In our study, seropositivity is obtained as 46.4%.

Our results imply that, good hygiene practices are essential to prevent *H. pylori* transmission for those in frequent contact with the public. As moderate to heavy dental plaque emerged as a clear risk factor, reducing plaque may have an important role in preventing acquisition of *H. pylori*. This is another reason for educating the public, adults and children about effective tooth-brushing and other oral hygiene techniques. Regular dental controls need to be encouraged, while avoiding the ingestion of plaque debris which could occur during tooth scaling. In view of these potentially important public health implications, confirmatory evidence of our results should be sought.

Some investigators suggested that dental plaque may be a reservoir for *H. pylori*, which is probably transmitted by person-to-
person contact, and blood group B antigen may predispose to infection. Some authors suggested that more comprehensive studies are needed to determine, whether dental plaque is an important reservoir in the epidemiology of *H. pylori* induced gastric disease (26, 28, 29, 30).

Although Buth et al. stated that, the prevalence of *H. pylori* in dental plaque of patients with dyspepsia was very high, our study indicates it to be a major reservoir of infection. But we think that our results strongly suggest that, oral to oral route is not an important mode of transmission in the adult population. Although dental plaque has a mixed flora that might act as a reservoir for gastric reinfection, dental plaque could not be implicated as the major reservoir of *H. pylori* for gastric reinfection. Luman et al. arguments are parallel to ours.

Community education about effective oral hygiene, and adoption of good hygiene practices by those with regular public contact is essential to prevent acquisition and transmission of *H. pylori*.

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REFERENCES