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ORIGINAL ARTICLE

Comparison of different methods to detect *Helicobacter pylori* in the dental plaque of dyspeptic patients

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Abstract The aim of this study was to compare different methods of detection of *Helicobacter pylori* (*H. pylori*) in the dental plaque of dyspeptic patients. After recording the clinical indices, culture and polymerase chain reaction (PCR) methods were performed on plaque samples, while rapid urease test in addition to these tests was carried on gastric samples from 67 dyspeptic patients who attended for an upper gastrointestinal endoscopy. Forty-seven of 67 patients were *H. pylori*-positive in gastric biopsy material whereas the microbial dental plaque from 19 patients demonstrated *H. pylori* positivity detected by PCR. Among the patients, 25.4% harbored *H. pylori* both in the stomach and in microbial dental plaque. No significant correlations were found among the presence of *H. pylori* in the stomach, in plaque, and clinical variables ($P>0.05$). Although oral hygiene was observed optimal and the mean of pocket depth was not found to be higher, the prevalence of *H. pylori* was observed to be higher in dental plaque. According to our results, PCR technique gave the highest detection rate both in gastric biopsy and in dental plaque compared to the other methods used.

Keywords *Helicobacter pylori* · Microbial dental plaque · Polymerase chain reaction · Culture · Rapid urease test

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Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative, microaerophilic, motile bacterium associated with chronic gastritis and peptic ulcer [15, 18, 25]. There is little knowledge about how *H. pylori* gastric infection is acquired, its reservoir, and its route of transmission. This microorganism may be transmitted orally and has been detected in dental plaque, saliva, and feces [13, 17]. The potential of the oral cavity as a reservoir for gastric infection has been studied [5, 10]. In those studies culture, histochemical staining, urease tests, and polymerase chain reaction (PCR) were used to identify this bacterium.

The oral cavity of patients with gastritis could be a reservoir for spread of the disease if *H. pylori* is present. It would also be a likely source of reinfection, accounting for recurrence of *Helicobacter* gastritis and associated duodenal disease in 35% of patients with duodenal ulcer in the year after eradication of infection by systemic antibiotic therapy [21]. In a recent study by Gebara et al. [11], 60% of the patients who underwent triple therapy (lansoprazole, 30 mg; amoxicillin, 1 g; and clarithromycin, 500 mg, two times a day for 7 days) were positive for *H. pylori* DNA in their oral cavity after the treatment [11].

The reports on the prevalence of *H. pylori* in the mouth and in the gastric mucosa of adult dyspeptic patients are controversial and the importance of oral hygiene in the colonization of this bacterium has not been completely understood [6]. It was found out in a recent case control study that the periodontal disease status and poor oral hygiene may not be important risk factors for *H. pylori* infection [2].

Moreover, although some authors reported higher prevalence of *H. pylori* in the oral cavity [9], supragingival dental plaque and saliva were not found as relevant reservoirs of *H. pylori* [14, 20].

One of the reasons for the disagreement found in the literature may be the different methodologies used to detect *H. pylori*. Therefore, the aim of this study was to detect the presence of *H. pylori* in dental plaque and in gastric biopsy samples, and to compare the different methods used for identification.

Materials and methods

Study population

Sixty-seven patients who had dyspeptic complaints were recruited for this study and asked to give an informed consent to participate after a detailed explanation of the procedures and objectives of the study. All were otherwise healthy and had not taken anti-inflammatory agents, antibiotics, immunosuppressants, or systemic contraceptives in the past 6 months. Selected patients had at least 18 teeth including two upper contralateral molars with probing depth ≤3 mm. Exclusion criteria were the use of antimicrobials, inhibitors of proton bomb, H_2 blockers, and bismuth derivates within 2 months before the clinical protocol; previous eradication therapy; upper digestive hemorrhage; pregnancy; and breast feeding [11].

The patient group included 34 women (mean age=41.03±13.68 years) and 33 men (mean age=39.74±12.05 years). Periodontal disease status was determined by clinical periodontal assessments, including plaque index (PI) [22], gingival bleeding index (GBI) [1], probing pocket depth (PPD), and clinical attachment level (CAL). The clinical measurements were obtained using a Fox–Williams periodontal probe to the nearest 0.5 mm before gastric biopsy. All clinical measurements were recorded by one examiner (IT). Recruited patients were diagnosed to be periodontally healthy (Table 1).

Subgingival plaque sampling was performed by curette before any periodontal treatment. Supragingival plaque from upper molar region was removed in conjunction with record of PI and subgingival plaque was collected by isolating the area with cotton rolls and drying the teeth and adjacent marginal gingiva with air. Samples contaminated by saliva or blood were excluded. Samples containing gastric biopsy were collected by a physician from patients with dyspeptic complaints.

Table 1 Means of clinical variables in *H. pylori*-positive and *H. pylori*-negative patients in dental plaque and in gastric biopsy samples

Clinical variables	*H. pylori*-positive (mean±SD)	*H. pylori*-negative (mean±SD)	*P* values
Dental plaque samples			
Pocket depth (mm)	2.76±1.24	2.48±1.2	0.555
CAL (mm)	2.85±1.41	2.63±1.2	0.648
PI	1.17±0.85	0.85±0.67	0.273
GBI (%)	23.36±29.65	12.31±10.57	0.143
Gastric biopsy samples			
Pocket depth (mm)	2.68±0.93	2.37±1.66	0.516
CAL (mm)	2.79±1.06	2.53±1.63	0.597
PI	1.09±0.76	0.67±0.65	0.145
GBI (%)	19.6±22.18	8.77±8.93	0.151

No significant differences $P>0.05$

Urease test was performed at least 2 h after the endoscopy procedure. The specimens were placed in brain hearth infusion (BHI) broth (Oxoid), and transported to the microbiology laboratory within 3 h.

Isolation of *H. pylori*

The specimens were inoculated on BHI agar containing 7% horse blood and *H. pylori*-selective supplement (Oxoid-SR 147E) and then incubated under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2) at 37°C for 3–7 days. The bacteria were identified as *H. pylori* based on colony morphology, Gram stain, motility, and production of urease, catalase, and oxidase reactions. *H. pylori* NCTC 11637 was used as the reference strain. All strains were stored in microcentrifuge tubes containing skim milk at −70°C until *urease A* PCR was done.

Amplification of *urease A*

Chromosomal DNA was extracted by the cetyltrimethylammonium bromide method according to the DNA Miniprep protocol of Wilson to determine the presence of *ureaseA* [26]. The primers HPU1 and HPU2 were used to amplify a 411-bp internal fragment of the *ureaseA* gene of *H. pylori* [8]. The primer set used for the detection of the *ureaseA* gene fragment from DNA was 5′GCCAATGG TAAATTAGTT3′ and 5′CTCCTTAATTGTTTTTAC3′. The PCR program for *urease A* gene was 95°C, 5 min, 1 cycle; 94°C, 1 min (denaturation), 45°C, 1 min (annealing), 72°C, and 1 min (polymerization) 35 cycles; and 72°C 5 min, 1 cycle. A 411-bp internal fragment of *urease A* gene was

Table 2 Pretreatment detection of *H. pylori* in gastric and microbial dental plaque samples of 67 subjects by conventional biopsy-based tests, culture, and PCR

Method of detection (sample)	Number tested	Number (%) positive
Microbial dental plaque samples		
Culture	67	–
PCR	67	19 (28.3)
Gastric biopsy samples		
RUT	67	28 (41.8)
Culture	67	27 (40.3)
PCR	67	47 (70.1)
RUT, culture, and PCR	67	54[a] (80.6)

RUT Rapid urease test, *PCR* polymerase chain reaction
[a] Samples were positive by any of that three tests.

amplified and the PCR products were resolved on a 1% agarose gel. *H. pylori* NCTC 11637 served as positive control and sterile distilled water was used as negative control. All samples were tested twice on different days.

Statistical analysis

Mean values and standard deviations for the clinical parameters were calculated with a statistical software package (Statistical Package for the Social Sciences, version 10.0 for Windows, SPSS Inc., Chicago, IL, USA). The dependence of the presence of *H. pylori* in the gastric biopsy and dental plaque was analyzed using the chi-square test. The correlations among clinical and microbiological parameters were analyzed using the Spearman correlation test. The criterion for statistical significance was $P<0.05$.

Results

The clinical indices for the entire mouth of 67 patients were evaluated. Oral hygiene was observed optimal and gingival bleeding was minimal in the patients (PI=0.95±0.73, GBI=15.99±19.37%). The mean of PPD was not found to be higher.

The means of clinical variables in *H. pylori*-positive and *H. pylori*-negative patients in dental plaque and gastric biopsy samples were given in Table 1. Although PI, GBI, and PPD was slightly higher in *H. pylori*-positive patients, nonsignificant differences were observed between *H. pylori*-positive and *H. pylori*-negative patients in both dental plaque and gastric biopsy samples ($P>0.05$).

Forty-seven of 67 patients were *H. pylori*-positive in gastric biopsy material detected by PCR whereas 27 samples were *H. pylori*-positive by culture method and 28 samples were *H. pylori*-positive by urease test (Table 2). The microbial dental plaque from 19 patients demonstrated *H. pylori* positivity according to PCR (Fig. 1). 25.4% of all patients harbored *H. pylori* both in the stomach and in microbial dental plaque. However, 55.2% demonstrated *H. pylori* positivity in stomach but not in microbial dental plaque. PCR has demonstrated the presence of *H. pylori* in the microbial dental plaque of 2.9% of patients whose gastric biopsies were negative for *H. pylori*.

The data indicated that PCR performed on gastric biopsy samples identified 24 samples, which were also culture-positive for *H. pylori*. PCR detected the presence of *H. pylori* in 18 patients, which were culture-negative. *H. pylori* was not isolated from any of the microbial dental plaque specimens with the culture method.

Discussion

H. pylori infection is one of the most common bacterial infections in man. The infection is widely accepted as an important cause of gastritis and is strongly associated with peptic ulcer disease and gastric cancer [12]. The human stomach was considered to be the only reservoir for *H. pylori* until bacteria were discovered in the human dental plaque, in oral lesions or ulcers, in oral cavity, and in saliva [17].

In the present study, PCR was found to have higher detection rates of *H. pylori* in gastric biopsy and in microbial dental plaque compared to that of other identification tests. Among them, in vitro urease tests are

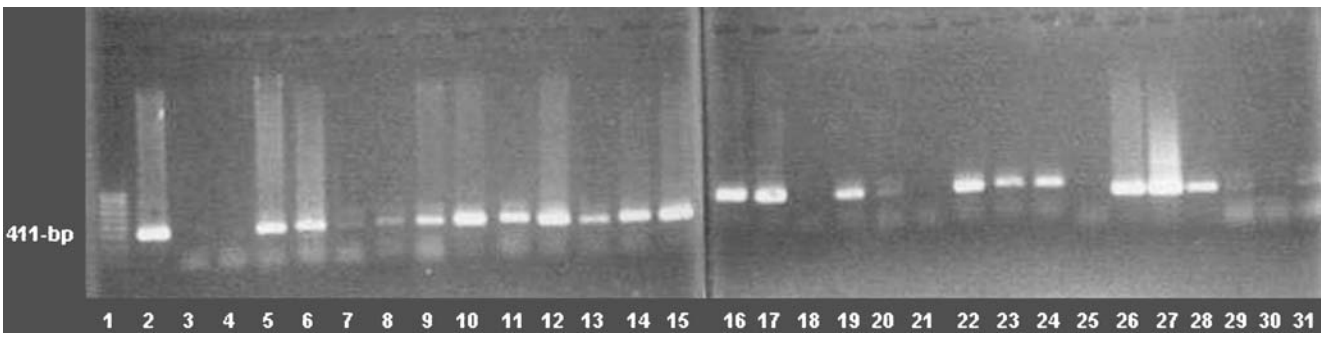

Fig. 1 Analysis of PCR products on an agarose electrophoresis gel. *Lane 1* DNA molecular weight marker, *lane 2* positive control (*H. pylori* NCTC 11637), *lane 3* negative control (ddH_2O as template), *lanes 4–31* microbial dental plaque samples of patients with dyspeptic complaints

dependent on the existence of urease in gastric biopsy specimens as *H. pylori* is the only urease-positive bacteria recovered from stomach. Conversely, urease-producing organisms are commonly found as part of the normal flora of oral cavity. *H. pylori*-like organisms were urease-, catalase-, and oxidase-positive and grew microaerophilically, but they were negative on *H. pylori*-specific PCR analysis, demonstrating the possibility of false identification [19]. For this reason, in the present study, urease test was not used for detection of *H. pylori* in microbial dental plaque because it was considered to be an unreliable test in the identification of *H. pylori* in microbial dental plaque.

In the present study, the attempts to culture *H. pylori* from the mouth were unsuccessful. This may be due to the organism being present in a nonculturable coccoid form [4, 16]. Our results were in accordance with a previous study reporting that *H. pylori* could not be found in the mouths of any of 94 patients, including 52 who had culture-positive gastric biopsies [4]. Therefore, more specific and sensitive methods are required for the detection of *H. pylori* in the oral cavity.

We found that 70.1% of all patients were gastric biopsy-positive and 28.3% were dental plaque-positive by PCR. Doré-Davin et al. [10] have reported that 22 patients enrolled in their study were infected by *H. pylori*; however, the presence of *H. pylori* was only detected in the oral cavity of 9 of them (41%) [10]. In another study, 97% of patients were reported to be *H. pylori*-positive in dental plaque sample; however, *H. pylori* DNA was detected in only 55% of the saliva samples [23]. The wide variations in the prevalence of *H. pylori* in the oral cavity probably originate from methodological differences among studies rather than from true geographical variations, i.e., in developing vs developed countries. Primers with different sensitivity and specificity and samples from different patient groups may also be responsible for these discrepancies [23]. Epidemiological studies have demonstrated that *H. pylori* infection is common in both developed and developing countries [23, 24].

In a previous study, it was shown that there was a specific distribution pattern for *H. pylori* in the oral cavity, with a higher prevalence in plaque from molars than from premolars or incisors [23]. Therefore, we performed sampling procedures from the molar region considering the microaerophilic characteristics of *H. pylori*.

The demonstration of the organism in the mouths of a substantial proportion of gastric patients has major implications for the spread of *H. pylori* and, in addition, the continued presence of *H. pylori* in the oral cavity may be an important source of gastric recurrence after eradication attempts. In a recent study, it was stated that viable *H. pylori* were present in gastric juice for potential transmission via the mouth [27].

In a previous study, where systemic antibiotics were applied to duodenal ulcer patients with gastric *H. pylori* infection, the oral cavity of patients were screened before and after antibiotic treatment [10]. It was reported that before treatment, 41% of infected patients harbored *H. pylori* in their mouth and that cure of the gastric infection did not promote the disappearance of *H. pylori* from the oral cavity of all patients [10].

The relationship between oral hygiene status and gastric and oral *H. pylori* presence was investigated in a recent study and *H. pylori* positivity was found to be correlated with poor oral hygiene scores; however, *H. pylori* was evaluated in dental plaque with campylobacter-like organism test [3, 7]. Because *H. pylori* is not the only urease-positive bacteria recovered from oral cavity and campylobacter-like organism test was not a reliable method to identify *H. pylori* in microbial dental plaque, the higher prevalence of *H. pylori* in the patients with poor oral hygiene might be due to false identification.

In the present study, no significant differences observed between *H. pylori*-positive and *H. pylori*-negative patients in both dental plaque and gastric biopsy samples. It was concluded that oral hygiene and gingival health might not affect the *H. pylori* colonization in Turkish patients. In fact, *H. pylori*-positive patients were expected to demonstrate poorer oral hygiene and more gingival bleeding compared to *H. pylori*-negative patients; however, according to our results, both groups demonstrated similar periodontal conditions. We considered that the relationship between oral hygiene and *H. pylori* colonization still remains to be a question and needs to be investigated in further studies.

PCR technique gave the highest detection rate both in gastric biopsy and in dental plaque compared to the other methods used in the present study, validating the conclusion that screening the patient after eradication of this bacteria could be well accomplished by PCR.

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