Effects of periodontal therapy on eradication and recurrence of Helicobacter pylori infection after successful treatment

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Abstract

Objectives: This study aimed to evaluate the effects of periodontal therapy on the efficacy of *Helicobacter pylori* eradication and on the recurrence of infection after eradication.

Methods: We conducted a prospective randomized trial on 698 gastric *H. pylori*-infected patients, of whom 347 received gastric *H. pylori* treatment alone and 342 received gastric *H. pylori* treatment plus periodontal therapy. The presence of *H. pylori* and associated virulence genes were detected by real-time polymerase chain reaction.

Results: After eradication of gastric *H. pylori* infection, the recurrence of gastric *H. pylori* was significantly lower in the gastric *H. pylori* treatment plus periodontal therapy group than in the group receiving gastric *H. pylori* treatment alone (OR 0.67; 95% CI 0.45 to 0.99), whereas the eradication rate was not significantly different (OR 0.87; 95% CI 0.68 to 0.98). There was a close relationship between the presence of *H. pylori* in saliva and its presence in the stomach.

Conclusions: The oral cavity is an important reservoir for gastric *H. pylori* infection. Adjunctive periodontal therapy could enhance the efficiency of *H. pylori* treatment and reduce the recurrence of gastric *H. pylori* infection.

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Introduction

Helicobacter pylori infection is an important gastrointestinal disease associated with the onset of gastritis, as well as gastric or duodenal ulcer disease and gastric cancer. Chronic gastritis-associated *H. pylori* infection has been found in approximately 50% of the global population and is etiologically linked to gastric cancers, comprising 25% of cancers related to this etiology of infection; therefore, it constitutes a primary public health problem.[1–4] In Thailand, the infection rate ranges from 54.1% to 76.1%;[5] however, the age-standardized incidence rate of gastric cancer is relatively low in Asian countries.[6] The progression of disease depends on multiple factors, which contribute to the onset of associated gastric diseases.[7]

Cytotoxin-associated antigen (*cagA*) and vacuolating cytotoxin (*vacA*) are the major virulence factors of *H. pylori*,[8] and are most closely associated with clinical outcomes of *H. pylori* infection.[9] The *cag* pathogenicity island (*cag* PAI) is a cluster of genes found in strains exhibiting enhanced interactions with gastric tissue.[9] This cluster encodes a bacterial type IV secretion system that translocates *cagA* into host gastric epithelial cells, and has been associated with the production of *vacA*, which causes cytoplasmic vacuolization in gastric epithelial cells;[9,10] moreover, it has been associated with delayed healing of gastric ulcers, inhibition of re-epithelialization, and worsening of the quality of mucosal scarring.[11] *H. pylori* strains harboring *cagA* and *vacA* are more frequent among patients with peptic ulcers, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancers.[12–14]

Although standard triple therapy regimens achieve successful eradication in *H. pylori*-positive gastritis patients, the recurrence rate is relatively high (13%).[16–18] Causes of gastric *H. pylori* recurrence-associated gastrointestinal diseases are not clear. There is a high prevalence of *H. pylori* DNA in dental plaque and saliva; notably, the oral cavity may constitute a reservoir for gastric infection and transmission.[18–22] Therefore, failed *H. pylori* eradication may be linked to the presence of oral *H. pylori*.

To determine whether eradication therapy alone or eradication therapy plus periodontal therapy contribute to reduced recurrence, it is necessary to identify factors involved in the recurrence of *H. pylori* in gastric mucosa. This study evaluated the association between the presence of *H. pylori* in saliva and its presence in the stomach, and investigated possible *H. pylori* treatment in Thailand. The findings of this study may improve treatment efficacy and reduce the risk of *H. pylori* recurrence in this region.

Patients and methods

Patients and sampling

Samples were obtained from patients diagnosed with *H. pylori*-associated gastric diseases who underwent esophagogastroduodenoscopy (EGD) at Suranaree University of Technology Hospital in the northeast region of Thailand from March 2017 to December 2017. Full-mouth periodontal examination was performed in all cases. The plaque index (PI) score was classified as follows: 0 = no plaque; 1 = a film of plaque adheres to the gingival margin, assessed by passing a probe across the tooth surface; 2 = moderate accumulation of plaque visible to the naked eye within the gingival pocket, and/or at the gingival margin and tooth surface; and 3 = abundant accumulation (1- to 2-mm thick) of plaque visible to the naked eye within the gingival pocket, and/or at the gingival margin and tooth surface. The gingival index (GI) score was recorded as follows: 0 = normal gingiva; 1 = mild

inflammation, slight change in color, slight edema, and no bleeding on probing (BOP); 2 = moderate inflammation, edema, redness and glazing, and BOP present; and 3 = severe inflammation, marked redness and ulceration, spontaneous bleeding, and edema. Probing depth (PD) and relative attachment level (RAL) were measured at six locations per tooth (mesial, distal, lingual/palatal, and buccal/labial) using a periodontal probe.[23–25] After baseline recording of clinical parameters, saliva was collected into a polypropylene tube, via the passive drool technique, until 2 mL of saliva was collected per patient; this saliva (2 mL) was mixed with DNAgard saliva stabilizer (1.5 mL; Biomatrica, San Diego, CA, USA), in accordance with the manufacturers' instructions. After oral clinical examination in accordance with the World Health Organization criteria, the patients were subjected to esophagogastroduodenoscopy (EGD), which was performed using an upper GI video endoscope (Olympus EVIS EXERA III, CV-190). First, the whole stomach was examined by conventional endoscopy; then, biopsies were conducted using the site-specific biopsy technique.[26] Gastric biopsy specimens for histological determinations were subsequently examined by the pathologist. Hematoxylin and eosin (H&E) and giemsa stains were used for *H. pylori* identification. All samples were stored at $-20°C$ until further processing by molecular methods.

H. pylori-associated gastritis patients, as determined by stool antigen, rapid urease test, or histological examination, were included in the study. The exclusion criteria were as follows: treatment for *H. pylori* infection during the previous 2 months; presence of metabolic disorders; immunosuppression; use of antibiotics or gastrointestinal medications, such as proton pump inhibitors, within the previous 2 months; previous gastric surgery; and a history of professional tooth cleaning or any other periodontal treatment within the past 6 months. We recruited a total of 689 *H. pylori*-associated gastritis patients who underwent EGD at Suranaree University of Technology Hospital (Figure 1). Demographic and clinical data, including sex, age, oral health status (dental caries, dental plaque, aphthous stomatitis, periodontitis, and dental caries + periodontitis) were collected for the enrolled patients. Written informed consent was obtained from all patients, and the study protocol was approved by the Ethics Committee for Research Involving Human Subjects, Suranaree University of Technology (EC 13-2560) and Thai Clinical Trials Registry (Study ID: TCTR20170324001). The methods were performed in accordance with the principles of good clinical practice and the Declaration of Helsinki guidelines.

Study groups

Six hundred eighty-nine *H. pylori*-associated gastritis patients were randomly divided into two groups using a random number generator from SPSS for Windows (version 16.0; SPSS Inc., Chicago, IL, USA) (Figure 1). Among these patients, 347 (168 female and 179 male; mean age of 46 ± 2 years; range, 17–80 years) were randomized to 1-week triple therapy (esomeprazole 20 mg twice per day, clarithromycin 500 mg twice per day, or metronidazole 400 mg three times per day (if clarithromycin-resistant), and amoxicillin 1000 mg twice per day (group 1)), and 342 patients (173 female and 169 male; mean age of 49 ± 3 years; range, 17–80 years) were randomized to triple therapy plus periodontal therapy (group 2). There were no significant differences between group 1 and group 2 in terms of numbers of patients, age, sex, or periodontal diseases.
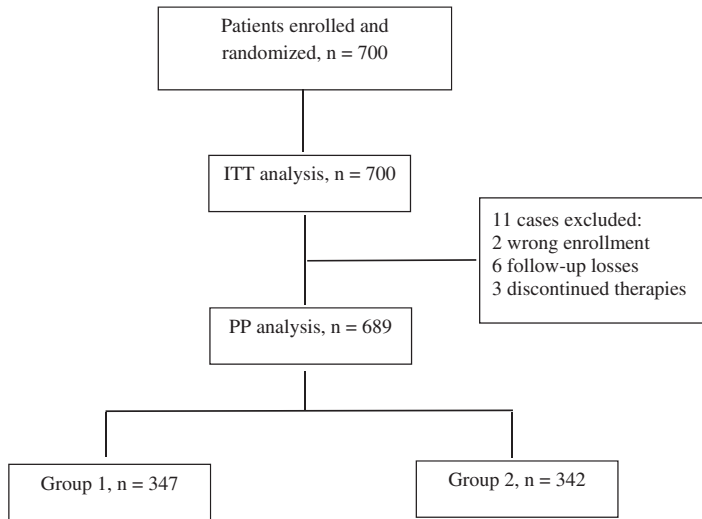
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                    ┌─────────────────────────┐
                    │  Patients enrolled and  │
                    │   randomized, n = 700   │
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                    │   ITT analysis, n = 700 │
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                                │                    │ 11 cases excluded:       │
                                │                    │ 2 wrong enrollment       │
                                │                    │ 6 follow-up losses       │
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                    │   PP analysis, n = 689  │
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          │ Group 1, n = 347  │   │ Group 2, n = 342  │
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Figure 1. Flow diagram showing numbers of patients enrolled and missed for per-protocol and intention-to-treat analyses. ITT: intention-to-treat; PP: per-protocol; ATB: antibiotic. Group 1: ATB alone, group 2: ATB plus periodontal therapy.

Follow-up statement

Patient follow-up was scheduled 4 weeks after the completion of therapy. Stool antigen tests, rapid urease tests, and histopathological examinations were performed as per routine practice, with polymerase chain reaction (PCR) for validation. Those who were negative for *H. pylori* underwent further examination after 1 year to establish the recurrence rate. Both invasive tests (gastric biopsy and PCR) and non-invasive tests (rapid urease test and histopathological examination) were used for diagnosis of reinfection with *H. pylori*.

Periodontal therapy

Scaling and root planning (SRP) is the gold standard in periodontal therapy. Treatment included hand instrumentation of the teeth to remove dental plaque and calculus from affected enamel and cementum, ultrasonic scaling therapy, flossing, irrigation with 0.12% chlorhexidine mouth rinse for 45 seconds, and oral hygiene instruction.[27] All patients were examined at 3 months

following SRP to confirm the therapeutic results.

DNA extraction

Genomic DNA was extracted from saliva and fresh frozen gastric biopsies using the QIAamp® DNA Microme Kit (Qiagen, Duesseldorf, Germany) and QIamp DNA mini kit (Qiagen), respectively, in accordance with the manufacturer's protocol. The extracted DNA concentration and purity were evaluated using a DS-11+ spectrophotometer (Denovix, Wilmington, DE, USA) and stored at -20°C.

Real-time polymerase chain reaction (PCR)

To determine the presence of *H. pylori* and its virulence genes, real-time PCR was performed. DNA extracted from saliva and gastric biopsies samples was used as template in the amplification reactions. Real-time PCR was performed in accordance with the manufacturer's protocol in a final

Table 1. Primer sequences used for real-time polymerase chain reaction.

Gene	Forward	Reverse
CagA	5′-GAGTCATAATGGCATAGAACCTGAA-3′	5′-TTGTGCAAGAAATTCCATGAAA-3′
VacA	5′-CTCCAGAAGGCACACCAATAA-3′	5′-TGGCTTCCACTTCCCCATTAA-3′
UreA	5′-CGTGGCAAGCATGATCCAT-3′	5′-GGGTATGCACGGTTACGAGTTT-3′
16s	5′-GGAGTACGGTCGCAAGATTAAA-3′	5′-CTAGCGGATTCTCTCAATGTCAA-3′

volume of 20 μl, containing cDNA template, 2X SYBR Green PCR Master Mix (Roche Applied Science, Mannheim, Germany), and 5 pmol of each primer using a LightCycler® 480 Instrument (Roche Diagnostics, Neuilly sur Seine, France). The PCR primers for *16s RNA*, *cagA*, *vacA*, and *ureA* (Integrated DNA Technologies, Coralville, IA, USA) are shown in Table 1. The PCR conditions were as follows: 95°C for 10 minutes, then 40 cycles of 95°C for 15 s and 60°C for 1 minute. All data were analyzed using LightCycler 480 software, version 1.5 (Roche Diagnostics).

Statistical analysis

All statistical analyses were performed using SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA). Comparisons between groups were performed using ANOVA for patient demographic data. Statistical significance of the correlations of the presence of *H. pylori* between saliva and gastric biopsies was evaluated using the χ^2 and Pearson's correlation tests. The eradication rates of *H. pylori* were evaluated by intention-to-treat (ITT) and per-protocol (PP) analysis. All patients were subjected to the ITT analysis; however, patients with incorrect enrollment, or who were lost to follow-up, were subjected to PP analysis. The association between treatment with *H. pylori* eradication therapy alone and *H. pylori* eradication therapy plus periodontal therapy was analyzed using a logistic regression model, with significance set at $p < 0.05$.

Results

Presence of H. pylori and virulence genes in saliva and stomach and their associations

Two *H. pylori*-specific genes, the *16S rRNA* and *ureA* genes, were included for *H. pylori* detection; these genes were detected in all 689 (100%) gastric biopsy samples, and were detected in 549/689 (79.7%) saliva samples. An association was observed between saliva *H. pylori* and gastric biopsy *H. pylori* ($p = 0.007$). Among the 549 *H. pylori*-positive cases, 231 were positive for the *cagA* gene (42%) and 223 were positive for the *vacA* gene (41%) in saliva. On gastric biopsy, 605 *H. pylori*-positive cases were positive for the *cagA* gene (87.8%) and 532 cases were positive for the *vacA* gene (77.2%). There were no significant associations between the virulence genes of *H. pylori* in saliva and gastric biopsy samples.

H. pylori eradication

We subsequently examined the potential therapy for gastric *H. pylori* eradication and its recurrence in patients with gastric *H. pylori* treatment alone and in those with gastric *H. pylori* treatment plus periodontal therapy. We found that gastric *H. pylori* treatment plus periodontal therapy produced a significantly lower recurrence rate of *H. pylori* infection than did gastric *H. pylori* treatment alone (OR 0.69; 95% CI 0.52 to 0.99; $p = 0.001$) by PP analysis; however, the eradication rates

Table 2. Outcomes of treatment with triple therapy alone and treatment with triple therapy plus periodontal therapy.

Variable	Treatment		OR (95% CI)	*p*-value
	ATB alone	ATB + periodontal therapy		
ITT				
Cure rate	304/350 (86.85)	331/350 (94.57)	0.87 (0.68–0.98)	0.076
Recurrence	53/350 (15.14)	7/350 (2)	0.67 (0.45–0.99)	0.001[*]
PP				
Cure rate	304/347 (87.60)	331/342 (96.78)	0.77 (0.58–0.97)	0.078
Recurrence	53/347 (15.27)	7/342 (2.04)	0.69 (0.52–0.99)	0.001[*]

ATB: antibiotic OR: odds ratio; CI: confidence interval; ITT: intention-to-treat; PP: per-protocol.

were not significantly different (OR 0.77; 95% CI 0.58 to 0.97) (Table 2). ITT analysis showed that the recurrence rates were significantly different between the groups (OR 0.67; 95% CI 0.45 to 0.99; $p = 0.001$), while the eradication rates were not significantly different (OR 0.87; 95% CI 0.68 to 0.98) (Table 2).

Discussion

H. pylori infection has been associated with chronic gastritis, peptic ulcers, gastric MALT lymphomas, and gastric adenocarcinomas.[28] Reported annual *H. pylori* recurrence rates after eradication therapy in developed and developing countries were 2.67% and 13.00%, respectively;[18] notably, the organism has been observed in various niches in the oral cavity, including the tongue, saliva, and dental plaque.[9,29] In Thailand, the reported annual recurrence rate of *H. pylori* infection after successful eradication was 2.9%.[30] To the best of our knowledge, this study is the first to determine the effect of periodontal therapy on gastric *H. pylori* eradication, as well as to evaluate the association between the presence of *H. pylori* in saliva and in the stomach in Thailand. We found that periodontal therapy plus gastric *H. pylori* treatment reduced the recurrence of gastric *H. pylori* infections. This result suggests a promising approach for reducing the risk of recurrent *H. pylori* infection. Moreover, we observed a significant association between *H. pylori* in saliva and gastric biopsy.

Avcu et al.[31] found that the recurrence of gastric *H. pylori* infection after triple therapy was more frequent among patients with poor oral hygiene than among patients with good oral hygiene. Furthermore, Adler et al.[32] demonstrated that the oral cavity was an initial extra-gastric reservoir for *H. pylori*; they found a strong relationship between *H. pylori* infections in the mouth and stomach. This observation may explain the consistently high recurrence rates of *H. pylori* infection in the stomach. Several studies have demonstrated that bacterial eradication in the oral cavity should be considered as an important aspect in the elimination of *H. pylori*-associated diseases, because the oral cavity may serve as a potential reservoir.[3,28,33–37] Our results agree with these observations, in that, after treatment for *H pylori* infection, patients treated with periodontal therapy plus triple therapy had a significantly lower recurrence rate of *H. pylori* infection, relative to the rate observed in patients who received only triple therapy. There was no significant difference in the eradication rates between the groups. Nevertheless, periodontal therapy plus triple therapy appears to have a better effect on

H. pylori eradication. We suspect that periodontal treatments may be useful adjuncts to triple therapy for *H. pylori* eradication.

Some authors have proposed that no living *H. pylori* can be found in the oral cavity and that the positive response on PCR may therefore result from regurgitation, vomiting, or reflux from the stomach; however, in the present study, we found *H. pylori* in the saliva and stomach of 79.7% and 100% of enrolled patients, respectively ($p = 0.007$). We speculate that the oral cavity is a potential reservoir for *H. pylori* and may be the source of gastric infection (and re-infection) and bacterial transmission. Currently, it remains unclear whether *H. pylori* strains in the oral cavity are the same strains that cause gastritis. Nevertheless, genotyping of *H. pylori* strains isolated from the stomach and the oral cavity have demonstrated that strains from these two sites appear to be identical, although different strains are harbored by different individuals.[9,38] Additionally, some studies have shown 98% agreement between DNA sequences of *H. pylori* in the stomach and corresponding strains in dental plaque or saliva,[9] suggesting that at least some individuals harbor the same strain in both the stomach and the mouth.[9,28] There have been many reports that *H. pylori* strains carrying *cagA* and *vacA* are closely associated with the incidence of severe gastroduodenal diseases.[12–14] Therefore, *H. pylori* strains in the oral cavity that produce *cagA* and *vacA* may be considered highly virulent. We observed a high rate of detection of *H. pylori cagA*-producing virulent strains in saliva (42% positive), while 41% of strains produced *vacA* in saliva. This finding suggests that salivary *H. pylori* might be a risk factor for gastrointestinal reinfection. Interestingly, we observed no significant association between the virulence genes of *H. pylori* in saliva and those in gastric biopsy. It is possible that *H. pylori* from two different places may comprise the same strains. Therefore, genotyping of *H. pylori* strains isolated from stomach and saliva should be prioritized in future research.

Although our findings revealed an association between the presence of *H. pylori* in saliva and that in the stomach, the complete genomes of *H. pylori* strains in both sites should be sequenced, because this is the only method to assess genetic identity. Furthermore, additional multicenter studies and large-scale randomized controlled trials are required to provide a better representation of the actual data and evaluate the hypothesis.

Conclusions

To the best of our knowledge, this is the first study to assess the prevalence of saliva *H. pylori* strains in the northeast region of Thailand, where there is a high prevalence of *H. pylori* infection. An association was observed between *H. pylori* in saliva and in gastric biopsies, suggesting that the oral cavity might constitute a reservoir for gastric *H. pylori* infection and re-infection. We further observed that *H. pylori* eradication plus periodontal therapy reduced the recurrence of gastric *H. pylori* infection in both PP and ITT analyses. Therefore, periodontal therapy should be applied to reduce the risk of recurrence of *H. pylori* re-infection-associated gastritis.

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