Existence of *Helicobacter pylori* with low virulence rate in dental plaque and gastric mucosa of patients with periodontal disease

Abdolmajid Ghasemian[a], Hussein S. Al-Essa[b] and Raghed M. Jassem[b]

Objective: *Helicobacter pylori* is associated with gastric and peptic ulcer leading to gastric cancer progress. Gingival teeth grooves among patients with chronic periodontitis can act as reservoirs for *H. pylori* proliferation. The purpose of our study was assessment the association of *H. pylori* from dental plaques of patients with periodontitis with gastric colonization.

Methods: Among patients with periodontitis admitted to dentistry centers, 250 dental plaque and 250 gastric biopsy samples were obtained during 2016–2019. After bacterial identification, virulence genes including *cagA, cagT, cagE, vacA* and *hrgA* were screened using PCR technique.

Results: Fifty and 75 isolates were identified in periodontitis and biopsy specimens, respectively. In periodontitis strains, the rete of *cagA, cagT, cagE, vacA* and *hrgA* were as 18 (36%), 15 (30%), 14 (28%), 6 (12%) and 6 (12%), respectively. Among 75 biopsy strains, prevalence of *cagA, cagT, cagE, vacA* and *hrgA* were as 28 (34%), 24 (32%), 19 (25.3%), 11 (14.66%) and 7 (0.14%), respectively. There was higher rate of gastric ulcer among ages more than 45 years compared with age ranges 1–15 and 20–45 years ($P < 0.01$ and $P = 0.004$, respectively). No significant difference between men and women (35/75 vs. 40/75) was observed.

Conclusion: Although the prevalence of virulence genes was low among *H. pylori* strains from dental plaques, a relatively high-density of *H. pylori* among both sources was considerable. Accordingly, *H. pylori* possibly spread from dental plaque into gastric mucosa. Furthermore, the possible role of dental plaques among patients with periodontitis as sources for peptic ulcer by pathogenic *H. pylori* needs more in-depth verifications.

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Introduction

Helicobacter pylori is a Gram-negative, curved, oxidase-positive, microaerophilic and slow-growing bacterium colonized in gastric and duodenal epithelium of more than half of the world's population. Bacterial transmission routes include oral-oral or fecal-oral in which 58% of individuals have the bacterium in their gastric or mouth. Children are contaminated mostly through this route. Vacuolating toxin (vacA) is comprised of several polypeptides with 95 kDa including two A and B subunits [1]. Also, cytotoxin-associated gene A (*CagA*) encoded by *cag* pathogenicity island has crucial role in severity of infection and gastric ulcer. This virulence factor is transferred by type IV secretion system and is recognized as an oncoprotein owing to reducing the

[a]Department of Biology, Tehran Central Branch, Islamic Azad University, Tehran, Iran, and [b]College of Dentistry, Basrah, Iraq.
Correspondence to Abdolmajid Ghasemian, Department of Biology, Tehran Central Branch, Islamic Azad University, Tehran, Iran.
Tel: +98 9106806917; e-mail: majidghasemian86@gmail.com
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Table 1. Primer sequences for the *Helicobacter pylori* detection and virulence factors.

Gene	Primer sequences	Amplicon size (bp)	References
JW	F: 5-CGTTAGCTGCATTACTGGAGA-3 R:5-GAGCGCGTAGGCGGGATAGTC-3	259	[9]
cagE	F:5′-TTGAAAACTTCAAGGATAGGATAGAGC-3′ R:5′-GCCTAGCGTAATATCACCATTACCC-3′	329	[10]
cagA	F: 5′-AATACACCAACGCCTCCAAG-3′ R: 5′-TTGTTGGCGCTTGCTCTC-3′	499	[10]
cagT	F: 5′-GTGTTTTTAACCAAAGTATC-3′ R: 5′-CTATAGCCASTCTCTTTGCA-3′	842	[11]
hrgA	F: 5′-GTTGTCGTTGTTTTAATGAA-3′ R: 5′-GTCTTAAACCCCACGATTAAA-3′	594	[12]
vacA	F: 5′-GCCGATATGCAAATGAGCCGC-3′ R: 5′-CAATCGTGTGGGTTCTGGAGC-3′	259	[12]

apoptosis of epithelial cells [2,3]. *H. pylori* adheres to the epithelial cells via adhesins like sialic acid-binding adhesin and blood group antigen-binding adhesin to P120 cadherin and E-cadherin receptors [3]. In addition, duodenal ulcer promoting protein A (DupA) and outer inflammatory protein A (OipA) are other virulence factors [4,5]. There are several assumptions regarding the dental contamination with *H. pylori*, such as fecal–oral transmission and dentistry physicians contamination as carriers [6]. Early studies exhibited that *H. pylori* can bind to *Fusobacterium nucleatum* and *Porphyromonas gingivalis* in dental plaques [7]. This bacterium is also observed in saliva, gingiva plaque and onto the tongue surface. The bacterium is susceptible to gastric acidic conditions; methyl-accepting chemotaxis protein (TlpB) can participate in the resistance [8]. In this study, our hypothesis was an association between *H. pylori* in dental plaque among patients with periodontitis and gastric ulcer or carcinoma. The aim of the current study was *H. pylori* detection and virulence genes screening in dental plaque of patients with periodontal disease associated with peptic ulcer.

Methods

Bacterial isolates

Among 250 patients with periodontitis admitted to dentistry centers during 2016–2019, dental plaque samples were collected. The upper and lower parts of gingiva were sampled from each patient (three from each) using sterile curt and paper cone, respectively. Inclusion criteria were all patients with gastric disorders without age limitation according to endoscopic findings in the country but not foreign patients. Those patients with antibiotic consumption during a month prior to the study were excluded. A consent form for sampling was taken from each patient. Moreover, 250 biopsy samples were taken from same patients for determination of the probability of gastric infection with *H. pylori*. The samples were suspended into normal saline and cultured onto Brucella agar (Merk, Germany) supplemented with 1% starch and 5% CO_2 and incubated at 37 °C for up to 7 days. Any pinpoint colony like watery drop was adopted and subjected for biochemical (urease, nitrate, and oxidase and catalase production) and molecular identification tests.

DNA extraction

The bacterial total genome was extracted using Oiagen, (Hilden, Germany) DNA isolation kit according to manufacturer's instructions.

Detection of virulence factors

Common virulence genes including *cagA, cagT, cagE, vacA* and *hrgA* were screened using PCR technique and specific primers depicted in Tables 1 and 2. PCR conditions included 94 °C (3 min), followed by 30 cycles of 94 °C for 45 s, annealing (30 s), 72 °C for 1 min and final extension for 5 min. PCR products were visualized using 1% agarose gel (90 V and 30 mA).

Statistical analysis

Comparison of virulence genes-positive and negative strains among patients with periodontitis was conducted with SPSS version 20. *t* Test and analysis of variance were employed (significance level of <0.05).

Results

Patients and bacterial isolates

The mean age of patients was 41.5 ± 4.7 (range of 4–50 years). Of 250 dental plaque samples, 50 isolates were identified and confirmed using phenotypic and PCR tests. Of 50 patients, 22 (44%) were pediatrics lower than 12 years, three of which with gastric ulcer. Moreover, 75 isolates were identified from gastric biopsy samples. There

Table 2. The *Helicobacter pylori* virulence genes profiles from dental plaque and gastric mucosa.

Virulence genes profile/source	Dental plaque	Gastric biopsy
cagA/cagT/cagE/vacA/hrgA	5 (10%)	6 (8%)
cagA/cagT/cagE/vacA	5 (10%)	7 (9.33%)
cagA/cagT/cagE	14 (28%)	19 (25.3%)
cagA/cagT	15 (30%)	24 (32%)

was higher rate of gastric ulcer among ages more than 45 years compared with age ranges 1–15 and 20–45 years ($P < 0.01$ and $P = 0.004$, respectively). No significant difference between men and women (35/75 vs. 40/75) was observed.

Prevalence of virulence factors

Of 50 periodontitis strains, the rete of *cagA, cagT, cagE, vacA* and *hrgA* were as 18 (36%), 15 (30%), 14 (28%), 6 (12%) and 6 (12%), respectively. Among 75 strains from biopsy, prevalence of *cagA, cagT, cagE, vacA* and *hrgA* were as 28 (37%), 24 (32%), 19 (25.3%), 11 (14.66%) and 7 (0.14%), respectively. These findings exhibited that despite the existence of *H. pylori* in dental plaque of patients with periodontitis, they had low-pathogenicity rate. Noticeably, there was no significant association between *H. pylori* virulence factors and periodontitis.

Noticeably, three men and two women were colonized with *H. pylori* strains in both dental plaque and gastric mucosa sources which carried all the *cagA/cagT/cagE/vacA/hrgA* genes.

Discussion

Periodontal disease is a disorder caused following the infection and inflammation of the gingiva tissue and surrounding bones [3]. In the primary stages known as gingivitis, the tissue is hemorrhage, red and inflamed. Data have documented that mouth cavity can act as a reservoir or niche for *H. pylori* growth and bacterial transmission mostly through saliva secretions. *H. pylori* substantially causes gastric and duodenal cancers by expression of several virulence factors such as *cagA, vacA, dupA, oipA* and urease factors [13,14]. It is notable that those strains from gingivitis have also virulence factors which lead to dissemination of infection to gastrointestinal tract causing severe outcomes. These virulence factors act as markers of precancerous stage; hence, accurate control strategies must be conducted to inhibit cancer development. The *vacA* gene has high prevalence among isolates in various countries. We observed that 14% of strains from patients with gingivitis and 19% of those from gastric tissue carried *vacA* gene. Noticeably, data from dental plaques is scarce. Some predisposing factors such as low hygiene, genetics and bacterial factors have pivotal role in the development of infection [1]. In addition, *H. pylori* existence in mouth cavity maybe related to gastric-mouth reflex or enters from exogenous source. It has been clarified that this can be a marker due to resistant gastritis or recurrent infection. It is also worth considering that *H. pylori* with various virulence factors in mouth cavity exacerbate the gastric ulcer and lead to infection source even following treatment [15–17].

In our study, the mean age of patients was 41.5 ± 4.7 (range of 4–50 years). Of 250 dental plaque samples, 64 isolates in phenotypic test and 50 isolates in PCR were identified and confirmed. Of 50 patients, 22 (44%) were pediatrics lower than 12 years old 3 of which with gastric ulcer. Moreover, 75 isolates were identified from gastric biopsy samples. This highlights the importance of *H. pylori* in pediatrics necessitating surveillance and preventive strategies (26–29).

Of 50 periodontitis strains, the rete of *cagA, cagT, cagE, vacA* and *hrgA* were as 18 (36%), 15 (30%), 14 (28%), 6 (12%) and 6 (12%), respectively. Among 75 strains from biopsy, prevalence of *cagA, cagT, cagE, vacA* and *hrgA* were as 28 (37%), 24 (32%), 19 (25.3%), 11 (14.66%) and 7 (0.14%), respectively. These findings exhibited that despite the existence of *H. pylori* in dental plaque of patients with periodontitis, they had low-pathogenicity rate [17]. Some studies have proposed the association of dental plaque colonization and gastric cancer or gastric reinfection [18] and also determined various virulence factors [18,19]. Noticeably, there was no significant association between *H. pylori* virulence factors and periodontitis. Our study demonstrated a significant association between gastric ulcer and *H. pylori* colonization. We observed that three men (ages 45, 48 and 49 years) and two women (ages 33 and 41 years) were colonized with *H. pylori* strains in both dental plaque and gastric mucosa sources which carried the *cagA/cagT/cagE/vacA/hrgA* genes profile. These findings highlighted the colonization of more pathogenic *H. pylori* onto dental plaque among patients with gastric ulcers.

Conclusion

Although the prevalence of virulence genes was low among *H. pylori* strains from dental plaques, a relatively high-density of *H. pylori* among both sources was considerable. Accordingly, *H. pylori* possibly spread from dental plaque into gastric mucosa. Furthermore, the possible role of dental plaques among patients with periodontitis as sources for peptic ulcer by pathogenic *H. pylori* needs more in-depth verifications. Furthermore, dental plaques among patients with periodontitis act as sources of pathogenic *H. pylori* which can cause peptic ulcer. Most of patients with dental plaques had *H. pylori* in gingiva and gastric tissues. Our study demonstrated a significant association between gastric ulcer with *H. pylori* dental colonization.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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