ARCHIVES OF ORAL BIOLOGY 55 (2010) 896–901

available at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/aob

Presence of *Helicobacter pylori* in supragingival dental plaque of individuals with periodontal disease and upper gastric diseases

Denise G. Silva [a,*], *Roy H. Stevens* [b], *Jacyara M.B. Macedo* [c], *Rodolpho M. Albano* [c], *Marcio E.V. Falabella* [a], *Ricardo G. Fischer* [d], *Enno C. Veerman* [e], *Eduardo M.B. Tinoco* [a,d]

[a] *Escola de Odontologia, UNIGRANRIO, Duque de Caxias, Brazil*
[b] *Department of Endodontics, Maurice H. Kornberg School of Dentistry, Temple University, Philadelphia, PA, USA*
[c] *Departamento de Bioquímica, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, RJ, Brazil*
[d] *Departamento de Procedimentos Clínicos Integrados, Faculdade de Odontologia, Universidade do Estado do Rio de Janeiro, RJ, Brazil*
[e] *Department of Oral Biochemistry, ACTA, Amsterdam, Netherlands*

ARTICLE INFO

Article history:
Accepted 29 June 2010

Keywords:
Periodontitis
Helicobacter pylori
PCR
Dental plaque
Gastric disease

ABSTRACT

Background: *Helicobacter pylori* is a Gram-negative microorganism which is able to colonize the gastric mucosa and is associated with peptic ulcer, gastric carcinoma, and gastric mucosa-associated lymphoid tissue lymphoma. Several studies have detected this bacterium in the oral cavity, suggesting it as a potential reservoir. The aim of this study was to investigate the presence of *H. pylori* in the oral cavity of individuals with periodontal disease and gastric diseases.

Methods: 115 individuals, with mean age 49.6 (±5.8) years, were divided in 4 groups: (A) with gastric diseases and periodontal disease; (B) with gastric diseases and no periodontal disease; (C) without gastric diseases and without periodontal disease, (D) without gastric diseases and with periodontal disease. Supra and subgingival plaque samples were collected from posterior teeth of the individuals with sterile paper points, and prepared for Polymerase Chain Reaction analysis. Fisher's exact test was used for detecting statistical differences between groups ($p < 0.05$).

Results: *H. pylori* was detected in supragingival plaque of 9/36 (25%) of group A, 1/31 (0.3%) of group B, 0 (0%) of group C and 3/36 (8.3%) of group D. No subgingival samples were positive for *H. pylori*. There was a statistically higher prevalence of *H. pylori* in groups A and D when compared to B and C ($p < 0.05$).

Conclusion: *H. pylori* was detected in the supragingival plaque, but not in the subgingival plaque, of individuals with periodontal disease and upper gastric diseases. There was an association between the supragingival colonization of *H. pylori* and oral hygiene parameters such as the presence of plaque and gingival bleeding.

1. Background

Helicobacter pylori is a gram-negative bacterium that colonizes the gastric mucosa, and is associated with chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma. Chronic *H. pylori* infection is found in approximately one-half of the world's population and is etiologically linked to 63% of all stomach cancer or approximately 5.5% of the global cancer burden, and

* *Corresponding author*. Tel.: +55 2126673271; fax: +55 2126670591.
E-mail address: denisegomes@globo.com (D.G. Silva).
0003–9969/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.archoralbio.2010.06.018

approximately 25% of cancers associated with infectious etiology, and has therefore a major impact on public health.[1–4]

Recently, researchers have suggested that the primary extragastric reservoir for *H. pylori* is the oral cavity and may be the source of infection and transmission. Most of the studies that observed an oral condition as a factor in the promotion of extra gastric *H. pylori* activity examined dental plaque and saliva as a vehicle for *H. pylori* carriage.[5–7]

Dental plaque harbours at least 400 different bacterial species, and forms a biofilm in which organisms are intimately associated with each other and the solid substratum through binding and inclusion within an expolymer matrix, where coaggregation activity is a significant factor.[8] This biofilm adheres to supragingival and subgingival tooth surfaces, and it will quickly form in the absence of good oral hygiene measures, enhancing the survivability of some bacterias.[9] In periodontal lesions, the numbers of periodontopatic bacteria increase with the development of periodontitis and strains of *Porphyromonas gingivalis* and *Fusobacterum nucleatum* were found strongly coaggregated with *H. pylori* strains.[10,11] However, *Streptococcus mutans* and *Prevotella intermedia* have been shown to possess strong inhibitory growth activity against *H. pylori* strains in stab-cultures and caused the formation of the coccal form.[12]

Several studies have reported that the prevalence of *H. pylori* DNA in saliva and dental plaque may vary from 33% to 86%, which suggests the oral cavity as a potential reservoir for *H. pylori*.[13–18]

Although *H. pylori* was first isolated nearly 30 years ago,[19] the process of infection, reinfection or human transmission remains unclear and there are few reports of its prevalence in the oral cavity of individuals with periodontal disease and gastric diseases.[20] Since poor oral hygiene control is associated with higher levels of inflammatory periodontal conditions,[21] it seems biologically plausible and reasonable to investigate the presence of *H. pylori* in association with periodontal disease. Therefore, the aim of this study was to investigate the presence of *H. pylori* in the oral cavity of individuals with periodontal disease and gastric diseases.

2. Materials and methods

2.1. Study population

The population of this study consists of 115 individuals with complaints regarding the upper digestive tract, with or without periodontal disease, referred for treatment between January and June 2009, at the Medical Clinic of São José University Hospital, Nova Iguaçu, Brazil. The population consisted of 47 males and 68 females with mean age of 49.6 (sd = ±5.8), with no previous treatment for ulcer or gastritis. Smokers, individuals with systemic diseases other than gastric diseases, individuals who reported abusive consumption of alcohol, individuals who showed less than 20 natural teeth, and those who had received antibiotic treatment in the last 3 months, were excluded from the study. The presence of *H. pylori* and gastric disease was detected by biopsy's histology, using Giemsa modified technique, hematoxiline-eosine and Polymerase Chain Reaction (PCR). All clinical periodontal examinations were done by one experienced periodontist (DGS) using a periodontal probe (PUNC Hu-Friedy®, Chicago, USA) at six sites per tooth and included probing pocket depth and clinical attachment level, which was measured from the cementum–enamel junction (CEJ) to the bottom of the pocket. After the initial clinical periodontal evaluation, subjects were categorized as having or not periodontal disease. The diagnostic criteria for the presence of periodontal disease was the presence of at least 4 different teeth with periodontal pockets ≥5 mm and clinical attachment level >3 mm. Individuals with no signs of periodontal pockets after the clinical examination were included in the groups with no periodontal disease. No categorization of severity of periodontal disease was used in this study. Oral hygiene status was assessed in all individuals using a periodontal probe to detect the presence of visible plaque at the cervical portion of the buccal, mesial, lingual, and distal surfaces of each tooth. Gingival inflammation was evaluated by the means of dichotomous measures of bleeding, 30 s after probing to the bottom of the periodontal pocket or sulcus at the same six sites on each tooth on which probing pocket depth was measured.[22] All participants were divided in 4 groups:

(A) Individuals with gastric dispepsy and periodontal disease.
(B) Individuals with gastric dispepsy and no periodontal disease.
(C) Individuals without gastric dispepsy and no periodontal disease.
(D) Individuals without gastric dispepsy and with periodontal disease (Table 1).

The study was approved by the Scientific Research Ethical Committee of Pedro Ernesto University Hospital – Universidade Estadual do Rio de Janeiro and all participants signed an informed consent.

2.2. Sample collection

Sterile paper points were used to collect supragingival and subgingival plaque samples. Cotton rolls and air drying with syringe were used to avoid saliva contamination of the samples. Supragingival plaque samples from all groups were collected by placing the paper points on the lingual aspects of the molars. Subgingival samples from groups A and D, with periodontitis, were collected by inserting 3 sterile paper points in all pockets ≥5 mm for 10 s. All samples were then placed into separated, sterile Eppendorf tubes, which were then immediately placed in coolers containing dry ice for transportation and stored at −80 °C, until processed.

2.3. Processing of the supra and subgingival plaque

The samples were defrozen, dissolved in distilled water and centrifuged at 12,000 × *g* for 15 min. The supernatant was then discarded and the precipitate was kept on ice and received 500 μl of digestion buffer, containing 0.45% NP-40, 0.45% Tween 20, 10 mM Tris–HCL pH 8.3, 50 mM KCL, 1.5 mM $MgCl_2$ 0.06 mg/ml Proteinase K (Promega, Madison, WI, USA), for

Table 1 – Data from 115 individuals from groups A, B, C and D, showing, age range, distribution of males/females, the presence of *H. pylori* in supragingival plaque (%) mean percentage of sites with dental plaque, and mean percentage of sites with bleeding on probing.

Groups	N (age range)	Presence of *H. pylori* in supragingival plaque (%)	% of sites with plaque	% of sites with bleeding on probing
(A) With gastric disease and periodontal disease	36 (42–53 years) males = 13 females = 23	9 (25%)	83%	75%
(B) With gastric disease and without periodontal disease	31 (45–52 years) males = 15 females = 16	1 (0.3%)	54%	23%
(C) Without gastric and periodontal disease	22 (43–51 years) males = 9 females = 13	0 (0%)	32%	0%
(D) Without gastric disease and with periodontal disease	26 (45–58 years) males = 10 females = 16	3 (11.5%)	79%	71%
Total	115 (42–58 years)	13 (11.3%)		

solubilization. Incubation was done at 50 °C for 120 min and 100 °C for 5 min, and the tubes were centrifuged at 12,000 × *g* for 5 min. The samples were then precipitated with the same volume of isopropanol, 1/10 of the volume of sodium acetate 3 M pH 5.2 and incubated overnight at −20 °C. Centrifugation was done for 10 min at 12,000 × *g* and the supernatant was discarded. The samples were then washed with 500 μl of ethanol 70% (v/v), centrifuged for 2 min, and the supernatant was once again discarded. The precipitate containing nucleic acids were dissolved in 30 μl of sterile deionized water and stored at −20 °C until use.

2.4. PCR

DNA preparation of dental plaque samples of all individuals was subjected to PCR assay with specific primers. Ten microlitres of the processed samples was used for the amplification of the gene 16S rRNA, using two synthetic oligonucleotide primers containing 18 base pairs: HP1: 5′-TGG CAA TCA GCG TCA GGT AAT G-3′ and HP2-GCT-AAG AGA TCA GCC TAT GTC C-3′ (Oligos Etc., Wilsonville, OR, USA) according to the method described by Egstrand et al.[23] In brief, the reaction was carried out in 500 μl tubes containing 10 μl of 5× PCR buffer (Promega, Madison, WI, USA), 0.2 μM of dNTPs (Invitrogen, São Paulo, Brazil), 8 μl of each primer (36.5 ng/μl), 1.5 units of Tth DNA polymerase (Biotools, São Paulo, Brazil), and adjusted with sterile distilled water to 50 μl. The temperatures used in thermal cycler (PCR Express Thermal Cycler – THERMO HYBAID, Ashford, Middlessex, UK) were 94 °C for 5 min for the initial denaturation, followed by 45 amplification cycles at 94 °C, 55 °C and 72 °C for 1 min each and followed by 72 °C for 5 min for the final extension. When the reaction was finished, the final product was stored at 4 °C until it was analysed. The products were separated by agarose gel electroforesis and stained with ethidium bromide. The 520 bp products were visualized on an ultraviolet light transiluminator (UVP – TM20, Ashfoord, UK; 13-K, Olympus, NY, USA).

2.5. Histological confirmation of *H. pylori* status and gastric disease

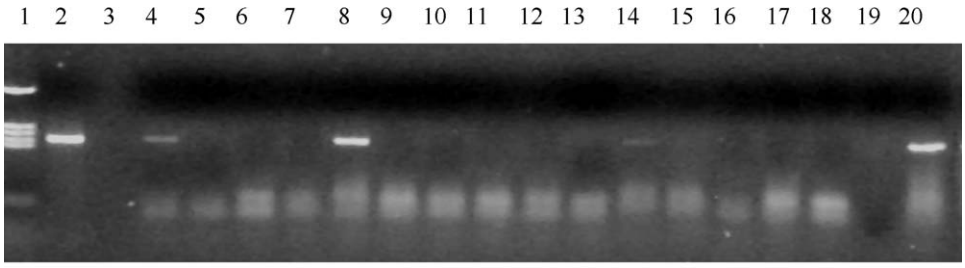
Gastric mucosal biopsy specimens were taken with jumbo forceps. Biopsy specimens, which were fixed in 10% buffered formalin for 48 h, were processed and embedded in paraffin wax. The paraffin blocks were sliced in serial sections of 4 μm, placed on slides, and stained by the modified Giemsa method and Hematoxylin–Eosine for optical microscopy analysis. The presence and density of *H. pylori* and the degree of inflammatory responses of the gastric mucosae were assessed and the results were scored as *H. pylori* infected or uninfected on the basis of the presence of the organism. The degree of inflammatory responses of the gastric mucosae was assessed on the basis of typical acute-or-chronic gastric disease.

2.6. Statistical analysis

Statistical analysis was performed by Fisher's exact tests, and Cochran–Mantel–Haenszel analyses.[24] The significance level was set at 0.05.

3. Results

There were significantly more individuals with sites showing bleeding on probing and plaque in groups A and D (with periodontal disease) than in groups B and C (Table 1; $p < 0.05$). All individuals (67) of groups A and B were confirmed to have gastric *H. pylori* infection by both histological and PCR of gastric biopsy specimens and displayed gastric mucosa inflammation compatible with the diagnosis of acute gastric disease. *H. pylori* DNA was detected in supragingival plaque of 9 (25%) individuals of group A (Fig. 1), 1 (0.3%) of group B, 0 of group C and 3 (11.5%) of group D. No subgingival samples were positive for *H. pylori* in any of the groups. There was a statistically higher prevalence of *H. pylori* in groups A and D when compared to B and C ($p < 0.05$). There was a statistically



Lane 1: 400 -bp ladder marker; **Lane2**: positive control *H. pylori DNA*; **Lane3**: negative control without DNA; **Lane 4 - 20**: dental plaque samples.

Fig. 1 – Representative agarose gel eletrophoresis of PCR products of dental plaque samples *H. pylori* positive.

higher prevalence of *H. pylori* in group A when compared with groups B and C ($p < 0.05$). There were no differences between males/females regarding *H. pylori* infection.

4. Discussion

H. pylory has been described as a key organism in the etiology of chronic gastritis, peptic ulcers[3] and gastric cancer,[2,4] and it's suppression and elimination has been considered the gold standard therapy for infectious gastric diseases.[25,26] Some authors have suggested that elimination of bacterium from the oral cavity should be regarded as an important part of the treatment of *H. pylory* associated diseases, since the oral cavity may serve as a temporary reservoir.[20–23] A recent study reported a higher *H. pylori* elimination rate when systemic triple therapy was used in combination with periodontal treatment (77.3%) compared to triple therapy alone (46.7%), suggesting that this could be a promising approach to increasing the therapy's efficacy and decreasing the risk of infection recurrence.[27] The reported proportion of *H. pylori* annual recurrence after eradication therapy is 2.67% and 13.00% in developed and developing countries,[28] and the organism has been found in different niches in the oral cavity, such as dental plaque, tongue, and saliva,[38–40] as well as in oral aftous ulcer lesions.[32] Haraszthy et al.[33] has also described that *H. pylori* DNA was detected by PCR in atheroma lesions of individuals with cardiovascular disease, and Mendall et al.[34] described the relation of *H. pylori* infection and coronary heart disease.

The results of the present study show that *H. pylori* was present in 13 (11.3%) supragingival plaque samples of individuals with or without periodontal disease independent of the infections of the stomach status. Similar results have been described by Song et al.[14] who reported the presence of *H. pylori* DNA in the supragingival plaque of individuals from developing countries. In order to colonize the gastro-intestinal tract the bacterium has to pass through the oral cavity where it is exposed to saliva and its components. Some of these components, such as mucins, may influence the colonization and the presence of *H. pylori* in the oral cavity, serving as specific receptors for the bacterium. We have previously shown that in some individuals higher levels of MUC5B in saliva were associated with the presence of *H. pylori*, suggesting that some individuals were at higher risk of harbouring the bacterium in the oral cavity.[35] The possible role of the oral cavity as a reservoir for *H. pylori* is a highly controversial issue,[36] and the role of saliva components and periodontal disease is still not elucidated. Most studies have examined supragingival or subgingival plaque from subjects without periodontal disease, showing a low detection rate.[16–19,29–31] The variation in results between those studies may be attributable to the different population groups examined and also the methods employed for plaque sampling, or specificity of primers.

The present study showed that *H. pylori* could be detected in the supragingival plaque of individuals in 3 different groups, but not in the subgingival plaque, suggesting that it does not frequently colonize the periodontal pocket. This is in agreement with the results from Asikainen et al.[36] who were not able to detect *H. pylori* in subgingival plaque of 336 patients with periodontitis, although no analysis for the presence of this bacterium in the stomach of those patients was performed in that study. On the other hand, other studies have shown *H. pylori* detection rates in subgingival samples from individuals with periodontitis ranging from 5.9% to 79%,[20–23] which suggests that some individuals may harbour the bacterium subgingivally. The use of periodontal curettes to collect plaque from subgingival pockets provides substantially more material to analyse than paper points, although contamination of the sample with supragingival material is almost unavoidable. Methodological variations and geographical differences between the studied populations most probably have a strong influence in the reported results.

Avcu et al.[37] observed that patients with poor oral hygiene were most likely to have *H. pylori* in their mouths and suggested that *H. pylori* could recur in the stomach of these patients after triple therapy more frequently than in patients with good oral hygiene. Genotyping of isolates obtained from both the oral cavity and stomach of patients have shown that strains from these two sites tend to be identical, although different strains are harboured by different individuals.[38,39]. In another study, we showed a 98% agreement between stomach *H. pylori* DNA sequence and their corresponding saliva or dental plaque DNA,[38] suggesting that at least some individuals harbour the same strain in the gut and in the mouth.

The results of the present study suggest an association between the supragingival colonization of *H. pylori* and oral hygiene parameters such as the presence of plaque and gingival bleeding. This is partially supported by the work of Jia et al.[40] who pointed out the importance of dental plaque as a temporary reservoir for the bacterium, and showed that dental

plaque control reduced the gastric infection by *H. pylori* in a Chinese population.

The cross-sectional design of the present study, and the limitations of statistical analysis when dealing with small samples and percentages equal to nil, implicate that inferences based on these results should be made with caution. Future longitudinal studies with larger samples can help elucidating which variables influence the presence of *H. pylori* in the mouth and what are the clinical implications of this infection.

5. Conclusions

H. pylori was detected in the supragingival plaque, but not in the subgingival plaque, of individuals with periodontal disease and upper gastric diseases. There was an association between the supragingival colonization of *H. pylori* and oral hygiene parameters such as the presence of plaque and gingival bleeding.

Funding

None.

Competing interests

The authors report no conflicts of interest related to this study.

Ethical approval

Ethical Approval was given by the Committee of Research Ethics from Hospital Universitário Pedro Ernesto (HUPE) under # 1509-CEP – HUPE.

REFERENCES

1. Forman D, Newel1 DG, Fullerton F, et al. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. *BMJ* 1991;**302**:1302–5.
2. Mbulaiteye SM, Hisada M, El-Omar EM. *Helicobacter pylori* associated global gastric cancer burden. *Front Biosci* 2009;**14**:1490–504.
3. Megraud F. Epidemiology of *Helicobacter pylori* infection. *Gastroenterol Clin North Am* 1993;**22**:73–88.
4. NIH Consensus Development Panel. *Helicobacter pylori* in peptic ulcer disease. *J Am Med Assoc* 1994; **272**:65–9.
5. Veerman EC, Bank CM, Namavar F, Appelmelk BJ, Bolscher JG, Nieuw Amerongen AV. Sulfated glycans on oral mucin as receptors for *Helicobacter pylori*. *Glycobiology* 1997;**7**:737–43.
6. Fritscher AM, Cherubini K, Chies J, Dias AC. Association between *Helicobacter pylori* and recurrent aphthous stomatitis in children and adolescents. *J Oral Pathol Med* 2004;**33**:129–32.
7. Alm RA, Bina J, Andrews BM, Doig P, Hancock REW, et al. Comparative genomics of *Helicobacter pylori*: analysis of the outer membrane protein families. *Infect Immun* 2000;**68**:4155–68.
8. Socransky SS, Haffajee AD. Microbial mechanisms in the pathogeness of destructive periodontal disease: a critical assessment. *J Periodontal Res* 1991;**26**:195–212.
9. Marquis RE. Oxygen metabolism, oxidative stress and acid–base physiology of dental plaque biofilms. *J Ind Microbiol* 1995;**15**:198–207.
10. Okuda K, Kimizuka R, Katakura A, Nakagawa T, Ishihara K. Ecological and immunopathological implications of oral bacteria in *Helicobacter pylori*-infected disease. *J Periodontol* 2003;**74**:123–8.
11. Andersen RN, Ganeshkumar N, Kolenbrander PE. *Helicobacter pylori* adheres selectively *Fusobacterium* spp.. *Oral Microbiol Immunol* 1998;**13**:51–4.
12. Ishihara K, Miura T, Kimizuka R, Ebihara Y, Mizuno Y, Okuda K. Oral bacteria inhibit *Helicobacter pylori* growth. *FEMS Microbiol Lett* 1997;**152**:355–61.
13. Banatvala N, Romero LC, Owen R, Abdi Y, Davies G, Hardie J, et al. Use the polymerase chain reaction to detect *Helicobacter pylori* in the dental plaque of healthy and syntomatic individuals. *Microbiol Ecol Health Dis* 1994;**7**:1–8.
14. Song Q, Lange T, Spahr A, Adler G, Bode G. Characteristic distribution pattern of *Helicobacter pylori* in dental plaque and saliva detected with nested PCR. *J Med Microbiol* 2000;**49**:349–53.
15. Mapstone NP, Lynch DA, Lewis FA, Axon AT, Tompkins DS, Dixon MF, et al. Identification of *Helicobacter pylori*-DNA in the mouths and stomachs of patients with gastritis, using PCR. *J Clin Pathol* 1993;**46**:540–3.
16. Özdemir A, Mas MR, Sahin S. Detection of *Helicobacter pylori* colonization in dental plaques and tongue scrapings of patients with chronic gastritis. *Quintessence Int* 2001;**32**: 131–4.
17. Bürgers R, Schneider-Brachert W, Reischl U, Behr A, Hiller KA, Lehn N, et al. *Helicobacter pylori* in human oral cavity and stomach. *Eur J Oral Sci* 2008;**116**:297–304.
18. Nguyen AMH, El-Zaatari FAK, Graham DY. *Helicobacter pylori* in the oral cavity. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1995;**79**:705–9.
19. Marshall BJ, Warren JR. Undentified curved bacilli on gastric epthelium in active chronic gastritis. *Lancet* 1983;**1**:1273–5.
20. Riggio MP, Lennon A. Identification by PCR of *Helicobacter pylori* in subgingival plaque of adult periodontitis patients. *J Med Microbiol* 1999;**48**(March (3)):317–22.
21. Anand PS, Nandakumar K, Shenoy KT. Are dental plaque, poor oral hygiene, and periodontal disease associated with Helicobacter pylori infection? *J Periodontol* 2006;**77**(April (4)):692–8.
22. Tinoco EMB, Beldi MI, Campedelli F, et al. Clinical and microbiological effects of adjunctive antibiotics in treatment of localized juvenile periodontitis. A controlled clinical trial. *J Periodontol* 1998;**69**:1355–63.
23. Egstrand L, Nguyen AM, Graham DY. Reverse transcription and polymerase chain reaction amplification of rRNA for detection of Helicobacter sp. *J Clin Microbiol* 1992;**30**: 2295–301.
24. Altman D. *Practical statistics for medical research*. New York: Chapman & Hall; 1991. p. 43–65.
25. Graham DY. Benefits from eradication of *Helicobacter pylori* infections include major reductions in the incidence of peptic ulcer disease, gastric cancer and primary gastric lymphoma. *Prev Med* 1994;**23**:712–6.
26. Bell GD, Powell KU. Eradication of *Helicobacter pylori* and its effect in peptic ulcer disease. *Scand J Gastroenterol* 1993;**28**:7–11.

27. Zaric S, Bojic B, Jankovic Lj, Dapcevic B, Popovic B, Cakic S, et al. Periodontal therapy improves gastric *Helicobacter pylori* eradication. *J Dent Res* 2009;**88**(October (10)):946–50.
28. Niv Y. *H. pylori* recurrence after successful eradication. *World J Gastroenterol* 2008;**14**:1477–8.
29. Oshwo A, Gillam D, Botha A, Tunio M, Holton J, Boulos P, et al. *Helicobacter pylori*: the mouth, stomach, and gut axis. *Ann Periodontol* 1998;**3**:276–8.
30. Dye BA, Kruszon-Moran D, McQuillan G. The relationship between periodontal disease attributes and *Helicobacter pylori* infection among adults in the United States. *Am J Public Health* 2002;**92**:1809–15.
31. Nguyen AMH, Engstrand L, Genta RM, Graham DY, El-Zaatari FAK. Detection of *Helicobacter pylori* in dental plaque by reverse-transcription polymerase chain reaction. *J Clin Microbiol* 1993;**31**(4):783–7.
32. Shimoyama T, Horie N, Kato T, Kaneko T, Komiyama K. *Helicobacter pylori* in oral ulcerations. *J Oral Sci* 2000;**42**:225–9.
33. Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. Identification of periodontal pathogens in atheromatous plaques. *J Periodontol* 2000;**71**:1554–60.
34. Mendall MA, Goggin PM, Molineaux N, Levy J. Relation of *Helicobacter pylori* infection and coronary heart disease. *Br Heart J* 1994;**71**:437–9.
35. Silva DG, Stevens RH, Macedo JM. Higher levels of salivary MUC5B and MUC7 in individuals with gastric diseases who harbor *Helicobacter pylori*. *Arch Oral Biol* 2009;**54**: 86–90.
36. Asikainen S, Chen C, Slots J. Absence of *Helicobacter pylori* in subgengival samples determined by polymerase chain reaction. *Oral Microbiol Immunol* 1994;**9**:318–20.
37. Avcu N, Avcu F, Beyan C, Ural A, Kaptan K, Ozyurt M. The relationship between gastric–oral *Helicobacter pylori* and oral hygiene in patients with vitamin B-12 deficiency anemia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001;**92**:166–9.
38. Silva DG, Stevens RH, Macedo JMB, Veerman E, Tinoco EMB. Detection of cytotoxin genotypes of *Helicobacter pylori* in stomach, saliva and dental plaque. *Arch Oral Biol* 2009;**54**:684–8.
39. Shames B, Krajden S, Fuksa M, Babida C, Penner JL. Evidence for the occurrence of the same strain of *Campylobacter pylori* in the stomach and dental plaque. *J Clin Microbiol* 1989;**27**:2849–50.
40. Jia CL, Jiang GS, Li CH, Li CR. Effect of dental plaque control on infection of *Helicobacter pylori* in gastric mucosa. *J Periodontol* 2009;**80**:1606–9.