Correlation of Helicobacter Pylon in Dental Plaque and Gastric Mucosa of Dyspeptic Patients

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Abstract

Objective: To establish the prevalence of Helicobacter pylon colonization of dental plaque and its correlation with Helicobacter pylon infection of the antral mucosa in patients with symptomatic dyspepsia.
Methods: Seventy eight adult dyspeptic patients undergoing upper gastrointestinal tract endoscopy were prospectively enrolled. Four air dried dental plaque cytology slides and four gastric antral mucosal biopsies were stained with Giemsa stain. CLO test was used for detection of urease activity of Helicobacter pylon in the dental plaque specimens and antral mucosal biopsies. Data on endsocopic findings and orodental hygiene were recorded.
Results: Dental plaque colonization using CLO test and cytology was found to be 100% and 88% respectively. Antral biopsy for H. pylon was positive in 61% cases by CLO test and 57% cases on histopathology. Forty four out of 69 patients (63%) had both dental plaque and antral biopsy positive for H. pylon. No patient with negative dental plaque cytology was positive for H. pylon in gastric mucosa. A statistically significant correlation was found between H. pylon colonization of dental plaque and gastric antrum. The sensitivity and specificity of dental plaque cytology in diagnosing H. pylon antral colonization was 100% and 26% while the positive and negative predictive values were 64% and 100% respectively.
Conclusion: The prevalence of H. pylon in dental plaque of patients with dyspepsia was very high in our patients indicating it to be a major reservoir of infection (JPMA 52:196;2002).

Introduction

Helicobacter pylor (H. pylori), a gram negative, curved, microaerophilic organism identified by Marshall and Warren in gastric antral mucosa is associated with chronic gastritis, peptic ulcer, non-ulcer dyspepsia, gastric carcinoma and mucosa associated lymphoid tissue (MALT) and B-cell MALT[1-3]. Human and animal antral mucosa were considered the only natural reservoirs of H. pylon until 1989 when the organism was recovered from dental plaque[4,5]. The mechanisms of oral colonization with H. pylon are unknown. The periodontal pocket with its microaerophilic acidic environment may be a permanent reservoir of H. pylor and could serve as a possible source of infection of the antral gastric mucosa[6-9]. Regional data on the prevalence of colonization of dental plaque by H. pylon is conflicting with very low figures from Western countries[10] while extremely high rates have been reported from developing Asian countries[11]. These differences could be a reflection of different diagnostic procedures used to identify H. pylon in these studies, existence of gingival or periodontal diseases and differing orodental hygiene practices of various ethnic groups. Reports on simultaneous H. pylon colonization of dental plaque and gastric antral mucosa are also

contradictory[12]. Studies by other workers have reported a very low prevalence of simultaneous colonization of dental plaque and gastric mucosa[9,13-15]. A strong correlation between oral carriage of H. pylon and gastric colonization was reported by other[6,7,16,19].
We have previously reported the prevalence of H. pylon in dental plaque of Pakistani patients[20]. An earlier study by Qureshi et al[21] from Karachi using CLO test reported 50% H. pylon plaque positivity in their cohort of 60 patients undergoing upper GI endoscopy. The present study was conducted at the Shaikh Zayed Hospital, Lahore to determine the prevalence of H. pylon in dental plaque of dyspeptic patients and to study the correlation between the presence of H. pylor in dental plaque and gastric antral mucosa of patients with symptomatic dyspepsia.

Patients and Methods

Seventy eight adult patients attending the gastroenterology outpatients department between October 1999 and September 2000 were prospectively enrolled in this study after obtaining informed consent. These patients had dyspeptic symptoms of sufficient severity to warrant an upper gastrointestinal endoscopy. Edentulous patients were excluded. Although there are no data suggesting the effect of drugs on oral carriage of H. pylon, patients who had used omeprazole, pencillins, tetracyclines, macrolides, quinolones, nitroimidazoles, bismuth and furazolidone that are likely to suppress H. pylon during 6 weeks preceding the study were excluded. We also excluded patients with diabetes, myxedema, chronic renal failure, cirrhosis, carcinoma esophagus and stomach and bleeding gastric or duodenal ulcers. Patients with ulcers but without evidence of any stigmata of recent bleed i.e. clot in ulcer bed, visible vessel or oozing from ulcer margins were included in the study.
An upper GI endoscopy was carried out under conscious sedation using 2.5 to 4 mg of intravenous midazolam and throat was anesthetized using a 10% lignocaine spray. After a thorough endoscopic examination, six antral biopsy specimens were obtained. Two antral biopsy specimens were immediately inoculated into CLO test gel (Delta West Co.,Australia). This test is based on the change in colour of the pH indicator when ammonia is generated from urea. The preformed urease of H. pylon acts on the substrate releasing ammonia and thereby increasing the pH to produce a colour change from yellow to redvoilet. Change in colour of the gel of CLO test and the time when it occurred were noted.
The remaining four antral biopsy specimens were preserved in formalin for histopathology examination using Giemsa stain.
Dental examination was undertaken by the dental surgeon; data were recorded on the state of oral hygiene, presence or absence of periodontal and gingival inflammation,denture use and the amount of plaque recorded (minimal, moderate or large). Oral hygiene was assessed as (1) good if there were no deposits and the gums were healthy, (2) fair in the presence of marginal gingivitis and bleeding on probing and (3) poor in the presence of visible deposits. All data were recorded according to the Community Periodontal Index of Treatment Needs (CPITN)22 and were scored as follows: 0 = healthy, 1 = maginal gingivitis, 2 = deposits, 3 = periodontal pocket of more than 4 mm but less than 6 mm, 4 = periodontal pocket of more than 6 mm A CPITN probe recommeded by WHO (JM-WHO, Yamaura Seisakusho Ltd., Japan) consisting of a ball ended probe and a black band marking at 4 mm and 6 mm was used to differentiate between scores 3 and 4. Gingival status was defined as marked gingivitis and inflammation when there was visible gingivitis that would bleed on touch with a CPITN score of above 1.
Six dental plaque (DP) specimens were obtained from lingual surface of the lower incisors and

buccal surface of the upper molars with a sickle sealer. The sickle sealer was inserted in the subgingival pocket and a single upward or downward stroke movement was used to extract the plaque. The instrument was autoclaved before every procedure. After collection, 2 dental plaque specimens were immediately inoculated into CLO test gel. Four remaining plaque specimens were used for preparing cytology slides. The plaque was placed between two clean glass slides that were gently pressed together and then pulled apart and dried in air. The air dried smears were stained with Giemsa stain23,24 and evaluated by two histopathologists who had no interest in the outcome of this study. H. pylon were identified when dark bluish-voilet stained curved, spiral shaped rods of approximately 2-3 mm in length were seen.

Facilities for detection of H. pylon by culture and PCR are not available at our centre. Detection of H. pylon was therefore based on histological examination and CLO test and smear'cytology and CLO test for the gastric antrum and dental plaque specimens respectively.

Statistical Analysis

Association between presence of H. pylon in gastric antrum and dental plaque and orodental hygiene and plaque colonization were evaluated using the Chi square test with Fisher's exact test where statistically appropriate.

Demonstration of H. pylon by histology in gastric antrum and cytology in dental plaque specimens was considered the gold standard for diagnosis. Significance was established at the 5% level.

Results

The results of this prospective study which included 78 adult patients with symptomatic dyspepsia are presented in Table.

Table. Time (Day) of first appearance of primary ossification centres in fore and hind limb long bones of Albino Rats.

Bone	Control	Experimental	Delay
Humerus	15.00±0.00	16.40±0.16	1.4±0.16
Ulna	15.00±0.00	16.20±0.20	1.2±0.20
Radius	15.00±0.00	16.00±0.16	1.0±0.10
Femur	15.40±0.16	16.90±0.23	1.5±0.16
Tibia	16.00±0.00	16.70±0.21	0.7±0.21
Fibula	16.00±0.00	17.50±0.21	1.5±0.16

Statistical Comparison

Bone	Groups	P value	S / N.S.
Humerus	C vs E	< 0.001	H.S.
Ulna	C vs E	< 0.001	H.S.
Radius	C vs E	< 0.001	H.S
Femur	C vs E	< 0.02	M.S.
Tibia	C vs E	< 0.01	M.S.
Fibula	C vs E	< 0.01	M.S.

Antral gastritis was the commonest abnormality found on endoscopy followed by equal number of cases with esophagitis/esophageal erosions and duodenal ulcers. Thirty five percent cases had a normal endoscopy. CLO test was found to be positive in all dental plaque specimens while it was positive in 61% of antral biopsies. Helicobacter pylon was demonstrated by dental plaque cytology in 88% cases and in 57% by histopathology in gastric antral specimens. All cases who had a

negative dental plaque cytology were also negative for H. pylon in gastric antrum. Sensitivity, specificity and positive and negative predictive values of dental plaque cytology in diagnosing H. pylon associated antral gastritis in patients with symptomatic dyspepsia were found to be 100%, 26%, 64% and 100% respectively.
We did not find any significant correlation between dental plaque cytology status and endoscopic findings. Similarly the correlation between plaque positivity and presence of gingival or periodontal inflammation was not present. However, a significant correlation was found between the amount of dental plaque and positive dental plaque cytology ($p < 0.05$). No significant sex differences were found for prevalence of H. pylon colonization in dental plaque or gastric antral mucosa.

Discussion

H. pylon is now acknowledged to play a critical role in acid-peptic related and neoplastic pathologies of the stomach and duodenum[1,2]. There are many uncertainties about the routes of transmission and possible sources of infection. Initially human and animal stomachs were regarded as the only natural reservoirs of infection. However, the identification of H. pylon in dental plaque and saliva suggested an oral-oral route of transmission. The human oral cavity has a very complex microflora with over 350 different species. Presence of carbohydrate fermenting organisms especially, Lactobacillus species, low oxidation-reduction potential and an optimum oral temperature of 35-37°C provide an ideal microaerophilic acidic milieu and a vast static area of unhealthy dental plaque for growth of H. pylon. This ecological niche also assumes great importance in terms of relapses of peptic ulcer disease in patients after an apparent successful eradication of H. pylon from gastric mucosa since triple drug therapy has been unsuccessful in eradicating H. pylon from dental plaque[25-27] . Oral cavity may therefore serve as a permanent extra gastric reservoir of infection.
Data on oral colonization of H. pylon is very variable and contradictory, probably a reflection of different diagnostic procedures utilized for identification[28,29]. Literature review also provides insufficient information on orodental hygiene, amount of plaque and the co-existence of gingival or periodontal disease with only two studies specifically addressing this issue[6,10].
Similarly contradictory results have been reported in the literature regarding simultaneous colonization of antral gastric mucosa and dental plaque with H. pylon. Bernander et al[13] did not find any dental plaque positive for H, pylon in 52 patients who had culture positive gastric biopsies. Luman and associates[9] did not find a single case of H. pylon positive dental plaque in their series of 120 adult dyspeptic patients. Cellini et aP[4] reported a single case of H. pylon positive dental plaque in their cohort of 24 patients who had H. pylon positive gastric biopsies. Cheng[15] did not find a single patient positive for H. pylon in dental plaque in their series of 122 patients who had gastroscopy for dyspepsia. Mapstone and colleagues[7], on the other hand, found that 5 out of 12 patients attending a dyspepsia clinic had evidence of oral carriage of H. pylon determined by PCR. Thirty eight percent patients with H. pylon in gastric biopsies reported by Nguyen et al6 had simultaneous dental plaque colonization with H. pylon. The study reported by Banatvala and associates16 demonstrated a 63% correlation between oral carriage of H. pylon and gastric colonization. Pytko-Polonczyk et al[17] reported that all of their 55 duodenal ulcer patients had H. pylon in dental plaque determined on CLO-test and culture. Similar results have been reported by Olsen et al[18] and Kopaanski et al[19] in patients with peptic ulcer disease.
The present study found a dental plaque prevalence of H. pylon of 100% and 88% determined by

dental plaque CLO test and dental plaque cytology, respectively. This is in conformity with results reported by Majmudhar[5] who used culture and CLO test and Desai1t who employed rapid urease test as the diagnostic method. D'Alessandro[28] reported greater than 70% dental plaque positivity in Italian patients using a combination of culture, biochemical and microscopic methods.
Identification of H. pylon in dental plaque using PCR has also yielded contradictory results varying from 86% in Bangladeshi children[30] to 38% in British patients[7]. This marked variation frobably reflects not only differences in methodology used but also the geographical location i.e. high prevalence in developing countries where H. pylon infection is acquired at an early age versus very low prevalence in Western countries where H. pylon infection is uncommon in childhood[31].
Dental plaque CLO test in our study became positive in patients in a mean time of 12.1 ± 9 minutes ($p > 0.05$). Studies carried out by Hazell[32] and McNulty[33] have demonstrated a good correlation between the rapidity with which CLO becomes positive and the overall density of H. pylon in the sample. Since all our patients had a positive CLO test very rapidly, we concluded that dental plaque in our patients had a very heavy density of H. pylori. However, it must be recognised that CLO test in dental plaque is not specific for H. pylon since other urease producing organisms most notably S. haemophilus, Bacteroides ureolyticus and Actinomycetes in dental plaque can also give a positive CLO test, but these are unlikely to do so within 1 hour[34].
Nguyen et al6 reported H. pylon in dental plaque of 39% of their patients but did not find any correlation with plaque deposits, gingival inflammation and oral hygiene. All of our patients with heavy plaque deposits were positive for H. pylon. Five patients in whom dental plaque was negative for H. pylon had minimal to moderate amount of plaque. The amount of dental plaque was correlated with dental plaque positivity ($p < 0.05$). We did not find any difference between positive plaque cytology and presence or absence of gingival and periodontal inflammation ($p > 0.05$). Interestingly, Peach et al[35] found a strong negative correlation between H. pylon status and having teeth scaled less than annually. They concluded that effective oral hygiene might be important in prevention and transmission of H. pylon.

Limitations of the study

We used CLO test, cytology and histology for demonstration of H. pylon in dental plaque and antral biopsy specimens. Facilities for PCR and culture were not available at our centre. These two later techniques that are more sensitive and specific would have provided information on whether the same strains of H. pylon were present in the dental plaque and gastric antrum.

Conclusion

The present study found a high prevalence of H. pylon colonization in dental plaque in adult patients with symptomatic dyspepsia compared to reports from the European centres. This difference could be a reflection not only of different diagnostic procedures but also due to differences in orodental hygiene, environmental factors and ethnic variations. The high prevalence of H. pylon in dental plaque and a statistically significant correlation between H. pylon in gastric biopsies and dental plaque could have important implications for the role of H. pylon in reinfection in patients with acid peptic disease after an apparently successful eradication of H. pylon from the stomach.

References

1.Forman D, Webb P, Parsonnet J. Helicobacter pylon and gastric cancer. Lancet, 1994;343:243-44.
2.Lambart JR. Lin SK, Aranda-Michel J. Helicobacter pylon. Scand J. Gastroenterol., 1995;30(suppl 208):33-46.
3.Bayerdroffer E, Neubauer A, Rudolph B, et al. Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue after cure of Helicobacter pylon infection. Lancet, I 995;345: 1591-4.
4.Krajden S, Fusak M, Anderson J, et at. Examination of human stomach biopsies, saliva and dental plaque for Campylobacter pylori J. Clin. Microbiol., 1989;27: 1397-98.
5.Majmudhar P. Shah SM, Dhunjbhoy KR, et at. Isolation of Helicobacter pylor from dental plaque in healthy volunteers. Ind, J. Gastroenterol., 1990;9:271-72.
6.Nguyen AMH, Engstrand L, Genta RM, et al. Detection of Helicobacter pylor in dental plaque by reverse transcription polymerase chain reaction. J. Clin. Microbiol., 1993,31:783-87.
7.Mapstone NP, Lynch DA, Lewis FA, et at. Identification of Helicobacter pylon DNA in the mouths and stomachs of patients with gastritis using PCR. J. Clin. Pathol,, 1993;46:540.43.
8.Cammarota G, Tursi A, Montalto M, et al. Role of dental plaque in the transmission of Helicobacter pylor infection. J. Clin. Gastroenterol., 1996;22:174-77.
9.Luman W, Alkout AM, Blackwell CC, et at. Helicobacter pylon in the mouth- negative isolation from dental plaque and saliva, Eur. J. Gastroenterol. Hepatol., 1996;8: 11-14.
10.Hardo PG, Tugnait A, Hassan F, et at. Helicobacter pylon infection and dental care. Gut., 1995;37:44-46.
11.Desai HG, Gill HH., Shankaran K, et al. Dental plaque: a permanent reservoir of Helicobacter pylori? Scand. J. Gastroenterol., 1991;26:1205-8.
12.Thomas E, Jiang C, Chi DS, et at. The role of the oral cavity in helicobacter pylon infection. Am. J. Gastroenterol., 1997;92:2148-54.
13.Bernander S, Dalen J, Gastrin B, et at. Absence of helicobacter pylon in dental plaques in Helicobacter pylon positive dyspeptic patients. Eur. J. Clin. Microbial. Infec. Dis., 1993;12:282-5.
14.Cellini L, Allocat N, Piattelli A, et al. Microbiological evidence of Helicobacter pylon from dental in dyspeptic patients. Microbiologica.,1995;18:187-92.
15.Cheng LHH, Webberley M, Evans M, et at. Helicobacter pylon in dental plaque and gastric mucosa. Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol.Endod., 1996,81:421-23.
16.Banatvala N, Romerolopez C, Owen RJ, et at. Use of the polymerrase chain reaction to detect Helicobacter pylon in the dental plaque of healthy and symptomatic individuals. Microbiol. Ecol. Health Dis., 1994;7:1-8.
17.Pytko-Poionczyk J, Konturek Si, Karezewska E, et at. Oral cavity as permanent source of Helicobacter pylon and potential source of reinfection. J. Physiol. Pharmacol., 1996;47:121-29.
18.Olsson K, Wadstrom T, Tyszkiewicz. H. pylori in dental plaques. Lancet, I 993;341 :956-7.
19.Kopaanski Z, Cienciala A, Banas J, et at. Coexistence of infection of the oral cavity and stomach and duodental mucosa with Helicobacter pylon in patients with ulcer and chronic gastritis. Wien-KIin Wochenschr., 1995;107:219-24 (German; English abstract).
20.Butt AK, Khan AA, Bedi R. Helicobacter pylor in dental plaque of Pakistanis. J. Int. Acad. Periodontol., 1999;l:78-82.
21.Qureshi H, Ahmad W, Arain G, et al. Correlation of histology, CLO, dental plaque and saliva in patients undergoing upper GI endoscopy. Am. J. Gastroenterol., 1999;94:861.
22.Ainamo J, Barnes D, Beagrie 0, et al. Development of the World Health Organization (WHO) Community Periodontal Index of Treatment Needs (CPITN). Int. Dent.J.,1982;32:281-91.

23.Mendoza ML, Martin-Rabadan P, Carrion I, et al. Helicobacter pylon infection: rapid diagnosis with brush cytology. Acta. Cytologica., 1993;37:181-5.
24.Price AB, The histological recognition of Helicobacter pylori. In Lee A McGraud F (eds.). Helicobacter pylon: techniques for clinical diagnosis and basic research. London: WB Saunders, l999,pp.33-49.
25.Pustorino R, Nicosia R D, Ambra 0, et al. The mouth-stomach crossing of Helicobacter pylori. Eur. Rev. Med. Pharmacol. Sci., 1996;18:183-6.
26.Perkins SE, Yan LL, Shen Z, et al. Use of PCR and culture to detect Helicobacter pylon in naturally infected cats following triple antimicrobial therapy. Antimicrob. Agents. Chemother., 1996;40: 1486-90.
27.Butt AK, Khan AA, Suleman BA, et al. Randomized clinical trial of Helicobacter pylon from dental plaque. Br. J. Surg., 2001 ;88:206.
28.D'Alessandro A, Ser S. Comparison of three different methods for evaluation of helicobacter pylon in human dental plaque. Bollettino-Societa Italians Biologia Sperimentale, 1992;68:769-73.
29.Madinier IM, Fosse TM, Monteil RA. Oral carriage of Helicobacter pylon: a review. J. Periodontol., 1997;68:2-6.
30.Banatvala N, Lopez CR, Owen R, et al. Helicobacter pylon in dental plaque. Lancet, 1993;341-80.
31.Pounder RE, Ng D. The prevalence of Helicobacter pylon in different countries. Aliment. Pharmacol. Ther., 1995;9:33-9.
32.Hazell S, Borody TG, Gal A, et al. Helicobacter pylon gastritis. I: detection of urease as a marker of bacterial colonization and gastritis. Am. J. Gastroenterol., 1987;82:292-96.
33.McNulty CAM, Wise R. Rapid diagnosis of Helicobacter pylori associated gastritis. Lancet, 1985;1:443-4.
34.Vaira D, Holton J, Cairns S, et al. Urease test for Helicobacter pylon: care in interpretation. J. Clin. Pathol., 1988;41:812-13.
35.Peach HG., Pearce DC, Farish SJ. Helicobacter pylon infection in an Australian regional city: Prevalence and risk factors. Med. J. Aust., 1997;67:3 10-13.