ORIGINAL ARTICLE

Molecular detection and corelation of *Helicobacter pylori* in dental plaque and gastric biopsies of dyspeptic patients

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ABSTRACT

Helicobacter pylori is a microaerophilic organism, which colonizes in the gastric mucosa. Its role in etiology and development of acute and chronic gastritis and peptic ulcer diseases is scientifically proved. Oral cavity especially supragingival, subgingival plaque and so forth simulate the same microaerophilic environment favorable for the growth of this bacterium. **Aim:** Detection of *H. pylori* simultaneously in the oral cavity and gastric mucosa of patients suffering from gastric pathologies. **Objectives:** To detect *H. pylori* in the oral cavity and gastric mucosa using endoscopy, urease test and real-time polymerase chain reaction (PCR) (urease A gene). Determining its association and corelation with patient demographics, oral hygiene maintenance and periodontal disease status. **Materials and Methods:** Endoscopic examination, oral findings oral hygiene index-simplified (OHI-S) and community periodontal index and treatment needs (CPITN) indices were recorded. Antral biopsies and supragingival plaque samples were taken from 56 dyspeptic adult patients. The collected samples were subjected to histological examination, urease broth test and urease A gene amplification using real-time PCR. **Result:** *H. pylori* was detected in the supragingival plaque of individuals with *H. pylori*-induced gastric diseases using rapid urease test and real-time PCR analysis. Occurrence of same strain of *H. pylori* simultaneously in plaque and gastric mucosa was observed. Positive correlation was obtained between the collected indices and quantity of *H. pylori* colonization.

Key words: *H. pylori*, real-time polymerase chain reaction, supragingival plaque, urease test

INTRODUCTION

Helicobacter pylori is a Gram-negative, microaerophilic, rod-shaped bacterium that inhabits the human stomach. It resides beneath the gastric mucous layer, adjacent to the gastric epithelial cells and causes inflammation of the gastric mucosa. Infection with this organism leads to serious transmissible infectious disease, linked to duodenal, gastric ulcers and gastric carcinoma.[1-3]

Researchers have suggested that the primary extra-gastric reservoir for *H. pylori* is the oral cavity, which may be the source of infection and transmission. Most of the studies demonstrated *H. pylori* within dental plaque and saliva, thereby making the oral cavity as an extra-gastric reservoir.[4-6] Dental plaque harbors at least 400 different bacterial species both pathogenic and nonpathogenic and forms a biofilm in which organisms are intimately associated with each other. In periodontitis, strains of *Porphyromonas gingivalis* and *Fusobacterum nucleatum* were found strongly coaggregated with *H. pylori*,[7,8] whereas *Streptococcus mutans* and *Prevotella intermedia* have been shown to possess strong inhibitory growth activity.[9]

H. pylori is also found in feces, so the route of infection could be oral-oral or fecal-oral. Because the oral cavity is an initial portal or gate to the gastrointestinal tract (GIT), microbial colonization and infection in the oral cavity may be associated with numerous stomach diseases. Although *H. pylori* was first isolated and identified nearly 30 years ago, the process of infection, reinfection or human transmission remains unclear. Several authors reported stating its prevalence and coexistence

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in the oral cavity and gastric mucosa of individuals, both with periodontal and gastric diseases. Because poor oral hygiene is associated with higher levels of inflammatory periodontal conditions, it seems reasonable to investigate the presence of *H. pylori* in association with periodontal disease.

The aim of this study was to detect *H. pylori* simultaneously in the dental plaque and gastric mucosa of patients suffering from gastric diseases and determine the association of *H. pylori* with oral hygiene maintenance and periodontal disease status.

MATERIALS AND METHODS

This study was carried out in Deparment of Oral and Maxillofacial Pathology, Periodontics, Department of Gastroenterology and Biotech Lab, Chennai. Study sample consisted of 56 patients (46 males and 10 females). Initially, 60 patients were selected of which four were excluded due to inadequate plaque sample. All patients belonged to same ethnic background and age range of 10-80 years with an average age range of 41-50 years. Sample selection was done based on inclusion and exclusion criteria. We included subjects with gastric problems who were advised for an endoscopic examination and on oral examination patients must have more than 10 teeth present. We excluded patients with drug history of any antimicrobial, proton pump inhibitors and H_2 blocker within 2 months before the study, also excluded patients with upper digestive hemorrhage and pregnant women. Informed written consent was obtained from subjects and study was approved by institutional ethical review committee. Vishnu Dental College, Bhimavaram, Andhra Pradesh, India.

The method in which the study was carried out included first clinical general examination and oral examination (to rule out less than 10 teeth). Endoscopic examination and three endoscopy biopsies were taken from patients of which one for histopathological conformation, one for urease test and one for polymerase chain reaction (PCR) analysis. After endoscopic evaluation and gastric biopsy collection, oral examination was done that included oral hygiene index-simplified (OHI-S) and community periodontal index and treatment needs (CPITN) indices were recorded. Supragingival plaque from posterior teeth was collected for urease test and PCR analysis. Urease test was done immediately in the laboratory but PCR analysis was done after 48 h.

Urease test is one of the diagnostic tests used to indicate the presence of urea-producing microorganisms. Procedure includes preparation of urease solution that consists predominantly urea and phenol red indicator. A total of 1 ml of this prepared urease solution was taken in a sterile warm test tube and the specimen was added into the preparation and incubated at 45°C for 5 min. Color change to pink indicates it is positive for urea-producing microrganisms and if the color does not change it is negative.

In the PCR laboratory, deoxyribonucleic acid (DNA) extraction and amplification was done. DNA extraction was done using real genomics YGB 100 DNA extraction kit, which consisted of reagents required for cell lysis and DNA isolation. Samples were subjected to these reagents, then GD column was used as the sieve to discard the flow through and remaining contents were placed back in the collection tube and allowed it to dry. This dried content was transferred to microcentrifuge tube and 5 µl of content was taken as template DNA to prepare 25 µl of PCR assay consisting master mix, primer and probe, template DNA and millipore water. This preparation is then transferred into the Eppendorf master cycler to run real-time PCR. The PCR was programmed to run 42 cycles with a standard cycle that consisted of annealing at 95°C for 15 s followed by extension at 60°C for 60 s. Sterile distilled water was used as non-template control and NCTC 11637 as positive control.

OBSERVATIONS AND RESULTS

Endoscopic findings of dyspeptic patients revealed 42.8% antral gastritis, 37.1% non-ulcer dyspepsis, 13.7% reflux esophagitis and 6.4% gastric carcinoma. The histological diagnosis was predominantly mild (26.7%), moderate-severe gastritis (41.9%), with few cases were acute gastritis (19.6%) and remaining gastric adenocarcinoma (11.8%). Urease test showed 80.4% of the gastric biopsies positive but only 19.6% plaque samples positive for urease-producing microorganisms [Table 1]. Real-time PCR findings showed presence or absence of urease A gene, which is specific for *H. pylori*, with average ct value for gastric biopsies was 35.87 and for dental plaque was 32.55 [Figure 1]. On statistical analysis of the age distribution, 55.3% of the patients belonged to 41-50 years and they had fair OHI-S with gingivitis. A total of 48.2% periodontitis recorded on CPITN index belonged to 30-50 years of age [Figure 2]. On statistical comparison between demographic data and PCR analysis, statistical association between *H. pylori* infection and age, sex and habit status of the patients showed insignificant result [Table 2 a-c]. Chi square comparative analysis on presence of *H. pylori* between urease test and PCR analysis, statistical significance existed with gastric biopsy and highly significant when supragingival plaque was analyzed, indicating a definite difference in efficiency between urease test and PCR analysis [Table 3]. On Spearman's rank correlation of oral hygiene and periodontal

Table 1: Comparison of the diagnostic methods for *H. pylori* (urease test and PCR analysis) in 56 dyspeptic peptic adults

Biopsy	*n* (%)		
	Urease test	PCR analysis	Total
H. pylori positive	45 (80.4)	34 (60.7)	79 (70.5)
H. pylori negative	11 (19.6)	22 (39.3)	33 (29.5)
Total	56 (100)	56 (100)	112 (100)

Chi-square (χ^2) value = 5.198; DF = 1; $P = 0.023$ significant.
PCR: Polymerase chain reaction

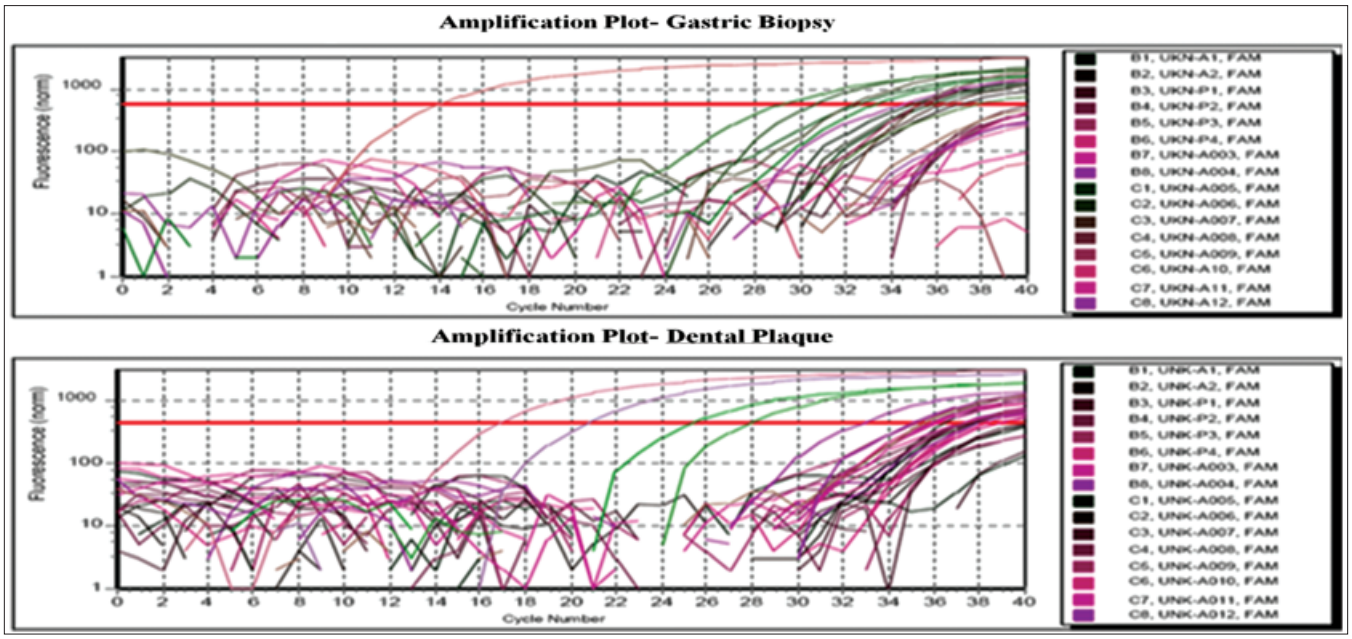

Figure 1: Amplification plot indicating CT values

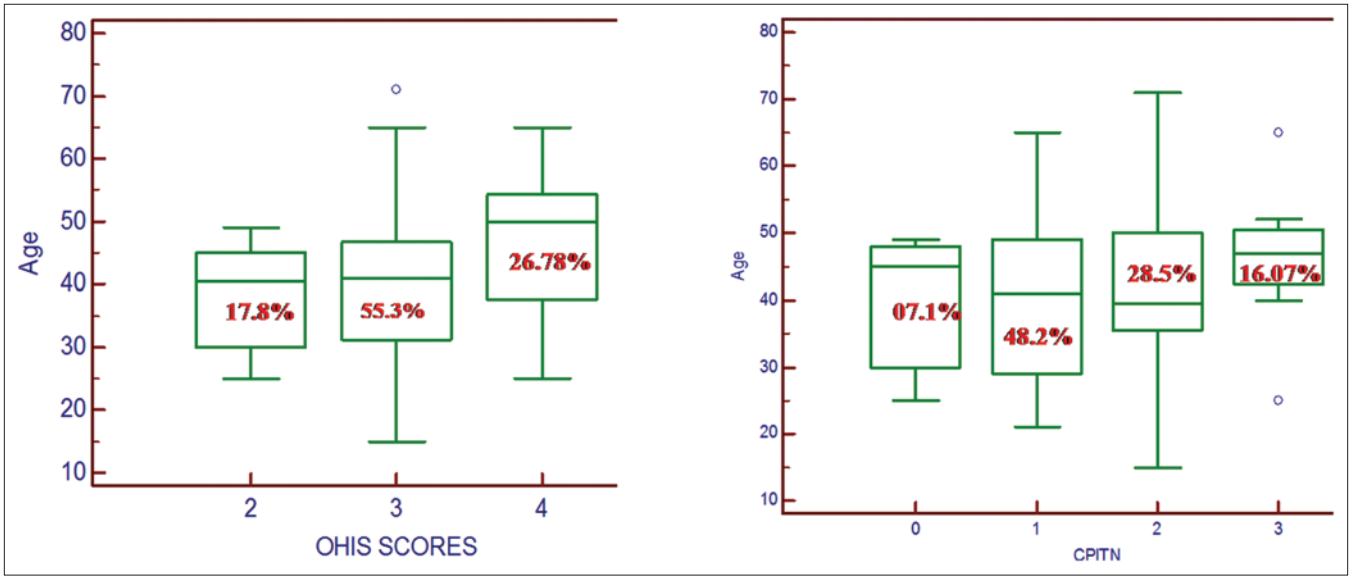

Figure 2: Correlation between the age of subjects and recorded indices

Table 2a: Correlation with age of the patient

Biopsy	*H. pylori* positive	34	42.79±12.27	0.606	0.548 NS
	H. pylori negative	22	40.68±13.03		
Plaque	*H. pylori* positive	19	38.89±10.00	−1.458	0.152 NS
	H. pylori negative	37	43.54±13.46		

Statistical analysis: unpaired *t* test. Statistically significant if $P < 0.05$.
NS: Statistically not significant

status with presence of *H. pylori* both in gastric biopsy and plaque, it was observed that a positive correlation existed [Tables 4 and 5].

Table 2b: Correlation with sex of the patient

PCR analysis	Sex *n* (%)		Total *n* (%)
	Male	Female	
Biopsy (+)	27 (58.7)	7 (70)	34 (60.7)
Biopsy (−)	19 (41.3)	3 (30)	22 (39.3)
Total *n* (%)	46 (100)	10 (100)	56 (100)
PLAQ (+)	12 (26.1)	7 (70)	19 (33.9)
PLAQ (−)	34 (73.9)	3 (30)	37 (66.1)
Total	46 (100)	10 (100)	56 (100)

Chi-square (χ^2) value = 0.440; DF = 1; $P = 0.507$ not significant.
PCR: Polymerase chain reaction

Table 2c: Correlation with habit status of the patient

PCR analysis method	Habit *n* (%)		Total
	Presence (score 1)	Absence (score 0)	
Biopsy (+)	9 (81.8)	25 (55.6)	34 (60.7)
Biopsy (−)	2 (18.2)	20 (4.4)	22 (39.3)
Total	11 (100)	45 (100)	56 (100)

Chi-square (χ^2) value = 2.556; DF = 1; $P = 0.110$ not significant.
PCR: Polymerase chain reaction

Table 3: Comparison of the efficacy of diagnostic methods used for *H. pylori* detection in supragingival plaque (urease test and PCR analysis)

Plaque	*n* (%)		
	Urease test	PCR analysis	Total
H. pylori positive	40 (71.4)	19 (33.9)	59 (52.7)
H. pylori negative	16 (28.6)	37 (66.1)	53 (47.3)
Total	56 (100)	56 (100)	112 (100)

Chi-square (χ^2) value = 15.795; DF = 1; $P < 0.0001$ highly significant.
PCR: Polymerase chain reaction

Table 4: Spearman's rank correlation coefficient between OHI-S score and presence of *H. pylori* in plaque

PCR analysis	Correlation coefficient (ρ)	*P* value	95% confidence interval for ρ
OHI-S-biopsy	0.0554	0.6848 NS	−0.211-0.314
OHI-S-plaque	0.468	0.0003 S	0.234-0.651

Statistically not significant if $P < 0.05$. Positive correlation.
PCR: Polymerase chain reaction; OHI-S: Oral hygiene index-simplified; NS: Statistically not significant

Table 5: Spearman's rank correlation coefficient between CPITN and presence of *H. pylori* in plaque

PCR analysis	Correlation coefficient (ρ)	*P* value	95% confidence interval for ρ
CPITN-biopsy	0.224	0.0976	−0.0418-0.459
CPITN-plaque	0.0912	0.5036	−0.176-0.346

Statistically not significant if $P < 0.05$. Positive correlation.
PCR: Polymerase chain reaction; CPITN: Community periodontal index and treatment needs

DISCUSSION

H. pylori was the first bacterium identified as a potential human carcinogen mainly on the basis of large seroepidemiologic case-control studies.[10-12] Its infection is ubiquitous involving 90% population of developing countries and proved to be a key organism in the etiology of chronic gastritis, peptic ulcer and gastric cancer[2,3,13] and its suppression and elimination has been considered the gold standard therapy for infectious gastric diseases.[14,15] Some authors have suggested that elimination of bacterium from the oral cavity should be regarded as an important part of the treatment of *H. pylori*-associated diseases, as the oral cavity may serve as a temporary reservoir.[16-19] A recent study reported a higher *H. pylori* elimination rate when systemic triple therapy was used in combination with periodontal treatment (77.3%) compared with 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. The reported proportion of *H. pylori* annual recurrence after eradication therapy is 2.67 and 13.00% in developed and developing countries, respectively.[20] The organism has been found in different niches in the oral cavity, such as dental plaque, tongue and saliva, as well as in oral aphthous ulcer lesions and it was reported to occur in childhood by oral ingestion and persisted for life in the host unless treated.[21-24]

Vakiland *et al*., stated that men were at higher risk when compared with that of women due to higher gastric acid secretion rates in men than women of same age and weight.[25] Russo *et al*., reported increase in prevalence of *H. pylori* and peptic ulcer disease among smokers.[26] But in our study, there was no association found between smoking and presence of *H. pylori* infection. Therefore, in our study there was no association found between the age, sex and smoking with the presence of *H. pylori* infection.

There are wide number and variety of invasive and non-invasive tests available for diagnosis of *H. pylori*. Among all tests it was reported that endoscopy and histological evaluation of multiple gastric biopsies remains as gold standard and in case of plaque molecular methods such as PCR. In our study, we used urease test and real-time PCR for detection of *H. pylori*. Urease test was chosen because it is most rapid and cost effective. Urease enzyme catalyzes the hydrolysis of urea to yield ammonia and carbamate. This carbamate spontaneously hydrolyzes to yield another molecule of ammonia and carbonic acid. Subsequently, ammonia equilibrates with water forming ammonia hydroxide and resulting in a rapid increase in pH. Thereby, changing the color of added indicator. Real-time PCR was chosen as it is accurate, highly sensitive and prepared primers probes are highly species specific.[27,28]

In the present study, we observed 72.8% sensitivity of urease test when compared with *H. pylori*-specific PCR results. The false-positive results were observed in urease test and it could be because of other urea-producing organism like *Proteus*, *Ureaplasma urealyticum*, *Nocardia*, *Cryptococcus* in GIT and *Streptococcus salivarius*, *Staphylococcus aureus* strains and *Diptheroids* strains in the oral cavity.[29] These organisms are also capable of reducing urea to ammonia thereby changing the color of the urease solution.

Anand *et al*., in 2006 reported periodontal disease status and poor oral hygiene may not be an important risk factor.[18] In our study, a positive corelation was found with oral *H. pylori* and recorded oral hygiene and periodontal health status indices, indicating that poor oral hygiene and diseased periodontal status creates a microenvironment conducive for the growth and replication of *H. pylori* organisms.

Literature studies reported the presence of *H. pylori* in the gastric mucosa and oral cavity, but we observed that plaque samples

of six out of 56 patients showed positivity for *H. pylori* and gastric mucosa samples negative for *H. pylori*. This could be because the patient must have undergone previous eradication therapy, wherein it completely eradicated gastric *H. pylori* but not plaque *H. pylori*. Also, Yamamura *et al*., stated that long-term nonsteroidal anti-inflammatory drugs (NSAIDs) therapy can also eradicate *H. pylori*, but it could lead to side effects like NSAIDs-induced gastric lesions.[30]

H. pylori eradication therapy includes administration of triple drug therapy, which includes antibiotics, antimicrobials and proton pump inhibitors. Subjects positive for oral *H. pylori* exhibit lower rate of eradication, as it is impossible to achieve effective concentrations of antibiotics from triple drug regime in saliva and dental plaque for eradication of oral *H. pylori*. Zanc *et al*., recommended an adjunctive treatment involving debridement of plaque along with triple drug regime. Eradication of oral *H. pylori* is effective in preventing the reappearance of gastric *H. pylori* infection from the oral cavity reservoirs.[31] In our study, patients with no history of eradication therapy for *H. pylori* were selected.

Therefore, we conclude that we detected the presence of *H. pylori* in supragingival plaque of individuals with *H. pylori*-induced gastric diseases. Same strain was isolated simultaneously from the plaque as well as gastric mucosa. On statistical analysis there was insignificant association found between demographic data and presence of *H. pylori* but a strong positive corelation was established between oral hygiene and periodontal disease status and presence of *H. pylori*. Periodontal therapy along with systemic triple drug regime must be practised in order to completely eradicate *H. pylori* from an individual. Presence of *H. pylori* in oral cavity is casual, but it is main causal for reappearance of gastric *H. pylori* infection.

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