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ORIGINAL ARTICLE

Study of the Coexistence of *Helicobacter pylori* and *Candida* in the Saliva of Patients with Dyspepsia

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ABSTRACT

Helicobacter pylori, a gram negative microaerophilic bacterium, is known to cause gastritis, peptic ulcer disease, and gastric adenocarcinoma. An extragastric reservoir of this bacterium can cause recurrent infection in treated patients. The oral cavity is considered an important extragastric reservoir owing to the favorable milieu in subgingival areas and the symbiotic association between *H. pylori* and *Candida*, a common commensal organism in the oral cavity. **Objectives:** To evaluate the presence of *H. pylori* and *Candida* in the saliva of patients with chronic gastritis. **Methods:** The study comprised subjects with *H. pylori*-positive gastritis (Group I $n = 30$), subjects with *H. pylori* negative gastritis (Group II, $n = 30$) and normal controls without gastritis (Group III, $n = 10$). Unstimulated saliva (whole mouth fluid) samples were collected and subjected to real-time polymerase chain reaction analysis for detecting the *Urease A* gene of *H. pylori*. Sabouraud's dextrose agar was used to detect the presence of *Candida* species. **Results:** Overall, 7 (23.3%) and 4 (13.3%) patients from Group I and Group II, respectively, were positive for oral *H. Pylori*, whereas 14 (46.6%) and 8 (26.6%) patients from Group I and Group II, respectively, exhibited oral *Candida*. Four patients (13.3%) from Group I exhibited both oral *H. pylori* and *Candida*. **Conclusion:** *H. pylori* and *Candida* in the oral cavity could potentially influence *H. pylori* recolonization of the gastric mucosa leading to recurrent gastritis.

Key words: *Candida*, dyspepsia, gastritis, *Helicobacter pylori*, polymerase chain reaction, saliva

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INTRODUCTION

Helicobacter pylori is a gram-negative microaerophilic, spiral bacterium measuring 2.5–5 μ in length. The organism has adapted to survive in the gastric mucosa. It contains flagella for facilitating motility in the viscous mucous lining of the stomach. The enzyme urease produced by the bacteria safeguards it against the gastric acidity and adhesin helps it to firmly bind to the mucosa. *H. pylori* infection can cause focal or multifocal atrophic or nonatrophic gastritis, peptic ulcer disease, noncardia gastric carcinoma, and lymphoma.^{1,2} *H. pylori* has been designated as a Group I carcinogen by the International Agency for Research on Cancer of the World Health Organization.³ Besides gastric mucosal diseases, *H. pylori* infection has been associated with other conditions including recurrent aphthous stomatitis,⁴ dyslipidemias,⁵ and coronary atherosclerosis.⁶

The natural niche of *H. pylori* is the gastric mucosa; however, extragastric sites, such as the oral cavity and esophagus,⁷⁻¹² may harbor this bacterium. In the oral cavity, *H. pylori* is commonly detected in the dental plaque,^{13,14} particularly surrounding the molar teeth.¹³ Moreover, *H. pylori* has been isolated from the tonsils, adenoids,¹⁵ and fingernails.¹² *H. pylori* is transmitted from person to person and colonizes the gastric mucosa. The persistence of *H. pylori* and resistance to treatment is attributed to the versatile adaptability of this organism via mutations and genetic recombinations between strains as well as via its ability to evade host immunity.¹⁶ Moreover, the persistence of this organism has been attributed to its presence in ecologically protected niches, such as the vacuoles of host immune cells and epithelial cells and within *Candida*.¹⁷ *Candida* is a ubiquitous fungi present in 40%–60% of healthy human oral cavities. Nonculturable bacterium-like bodies (BLBs) of *H. pylori* have been detected within

the vacuoles of *Candida* in the oral cavity and upper gastrointestinal tract of patients with peptic ulcer.¹⁹ *H. pylori* and *Candida* have a symbiotic relationship, which may have a role in the persistence of *H. pylori* within the oral cavity and gastric mucosa as well as in recurrent *H. pylori* infection.¹⁷

Numerous tests are available to detect *H. pylori*, such as the histopathological analysis of endoscopic biopsies, culture, Urea Breath Test (UBT), Rapid Urease test (RUT), enzyme immunoassay, Southern Blot, and polymerase chain reaction (PCR) of *H. pylori* genes, such as the cytotoxin vacuolating gene *Vac A s1 s2*,¹⁹ *16 S rRNA*,^{8,11,14,18} *Urease A*,^{9,10} and *Urease AB*.¹⁹

The present study was conducted to assess the presence of *H. pylori* and *Candida* in the oral cavity of patients with symptomatic gastritis.

METHODS

A total of 60 patients attending a gastroenterology unit for the endoscopic investigation of gastritis as well as the signs and symptoms of dyspepsia (complaints of abdominal pain, belching, retrosternal pain, and bloating), who were not undergoing any antifungal or proton pump inhibitor therapy, were included in the study. Moreover, 10 asymptomatic individuals without any history of gastritis or receiving any medication, who attended Ragas Dental College and Hospital, Chennai, for routine dental treatment, were included as controls. Based on endoscopic findings, the study participants were classified, as those with *H. pylori*-positive gastritis (Group I, $n = 30$), those with *H. pylori*-negative gastritis (Group II, $n = 30$), and the asymptomatic controls without gastritis and not undergoing any therapy (Group III, $n = 10$). A thorough history and physical examination findings were recorded in a preformatted questionnaire. Ethical permission was obtained from the Institutional Review Board of Ragas Dental College and Hospital, Chennai. Informed consent was obtained from the participants before enrollment in the study. Unstimulated saliva was obtained using the spit technique.⁸ The participants were instructed to spit (unstimulated) saliva into a 50 mL sterile container every minute for 10 minutes and the containers were stored in the deep freezer at -70°C at Ragas Dental College. The saliva sample of each patient was divided into two parts—one for PCR analysis and the other for *Candida* culture. DNA from the saliva samples was extracted by standard extraction protocol using the HiYield Genomic DNA Minikit – Real Genomics™ (Real Biotech Corporation, Taipei, Taiwan). Following DNA extraction, the DNA was quantified using spectrometric analysis to confirm the yield by monitoring the absorption (A) of UV light at various wavelengths—280, 260, and 230 nm. An absorption ratio at A260:A280 was performed to measure the contamination of proteins. An absorption

Figure 1: Amplification plot showing the positive samples crossing the threshold fluorescence. Samples crossing the threshold fluorescence are considered positive for *H. pylori*.

ratio of A260:A230 was performed to measure the contamination of phenolate ions, thiocyanates, and other organic compounds. All samples exhibited an A260:A230 ratio of >1.5 and A260:A280 ratio of >2.0 . The minimum yield of DNA required for amplification was $4 \text{ ng}/\mu\text{L}$. PCR was performed using the genomic DNA isolated from the saliva to detect the presence of *Urease A*. PCR amplification was performed in a total volume of $25 \mu\text{L}$ PCR assay using $12.5 \mu\text{L}$ Master Mix (Qiagen, Hilden, Germany), $4 \mu\text{L}$ Primer and Probe for *Urease A*, $5 \mu\text{L}$ template DNA, and $3.5 \mu\text{L}$ Millipore water. The recruited primers were as follows: Forward Sequence, GGC AAA AAA GCC GTT AGC GTG AA; Reverse Sequence, TGT CCC GCT CGC AAT GTC TAA G; and Probe Sequence, TTGGCGACAGACCGTTCAAATCGGCTCACAC. The PCR assay was transferred into Eppendorf PCR cuvettes and the cuvettes were centrifuged for 5 s and then transferred into Eppendorf Master Cycler® to perform real-time PCR. PCR amplification was programmed as follows: initial denaturation at 95°C for 15 min, followed by 40 cycles with a standard cycle containing denaturation at 95°C for 15 s followed by annealing and extension at 60°C for 1 min. The threshold line for the detection of fluorescence was fixed at 257 based on normalized data, and 40 cycles was set as the maximum. Samples crossing the fluorescence threshold line were considered positive and their cycle threshold (Ct) value was recorded (Figure 1).

For identifying the presence of *Candida*, the saliva collected in the sterile container was sampled using a sterile $0.001 \mu\text{L}$ loop and inoculated by streaking on Sabouraud's Dextrose Agar (HIMEDIA™, Mumbai, India) supplemented with chloramphenicol ($10 \text{ mg}/\text{mL}$) and incubated at 37°C for 48 h. After 48 h, the growth of white, creamy, smooth, raised *Candida* colonies along the line of streaking was recorded as a positive growth and the subject was considered as

Figure 2: Candidal colonies formed in Sabouraud dextrose agar medium after 48 h of incubation at 37°C.

a *Candida* carrier. The colony-forming units (CFU) were manually counted by multiplying the number of individual colonies by a factor of 1000, which was represented as CFU/mL (CFU/mL = No of colonies in a SDA plate $\times$ 1000) (Figure 2).

Statistical analysis

Statistical analysis was performed using SPSS™ version 11.0.1 (SPSS, Inc., Chicago, IL, USA). The χ^2 test was conducted to determine the age and sex distribution between the groups. Spearman correlation coefficient was used to assess the relationship between age, gastric and salivary *H. pylori*, and salivary *Candida*. Pearson χ^2 test was used to study the association between the salivary and gastric *H. pylori* and salivary *Candida*. Odds ratio (OR) and 95% confidence interval (CI) were estimated to study the association of salivary *H. pylori* and salivary *Candida* in the patients with gastritis. $P < 0.05$ was considered significant.

RESULTS

Overall, there were 23 males and 7 females in Group I (*H. pylori*-positive gastritis; 6 of these participants were <30 and 24 were >30 years old), 24 males and 6 females in Group II (*H. pylori*-negative gastritis; 3 of these participants were <30 and 27 were >30 years old), and 5 males and 5 females in Group III (8 of these participants were <30 and 2 were >30 years old. (Table 1).

Of the 60 patients with symptomatic gastritis, 11 (36.6%) exhibited *H. pylori* in the saliva [7/30 (23.3%) in Group I and 4/30 (13.3%) in Group 2; $p=0.5$]. No subject in Group III exhibited *H. pylori* in the saliva. The OR for gastritis and salivary *H. pylori* was 1.98 ($p=0.05$; 95% CI, 0.51–7.61). (Table 2)

Oral *Candida* was isolated from 22 patients with gastritis and from 3 participants in the control group. On comparison between the subjects in various groups, *Candida* occurred in the saliva in 14 participants in

Group I, 8 in Group II, and 3 in Group III ($P = 0.5$). (Table 3).

Significant correlation was not observed between the presence of gastric versus salivary *H. pylori* ($N = 60$, $r = 0.129$, $p = 0.3$), salivary *H. pylori* versus oral *Candida* ($N = 60$, $r = 0.003$, $p = 0.9$), and gastric *H. pylori* versus oral *Candida* ($N = 60$, $r = 0.208$, $p = 0.1$). No correlation was observed between overall age and candidal status of the saliva in patients with symptomatic gastritis ($p = 0.64$). On comparing the candidal status between the age groups in participants <30 years old, the OR was 7.5 (95% CI, 0.98–68.75, $p = 0.035$, Table 2). There was no significant difference in the mean CFU/mL between Groups I and II. Patients with gastritis exhibiting *H. pylori* and *Candida* in the saliva showed a mean CFU of 5250 ± 3095.69 . Patients who were positive for oral *Candida* and *H. pylori* in the gastric mucosa exhibited a 8.22 times higher risk of having symptomatic gastritis than those who did not have oral *Candida* (95% CI, 1.1–61.495, $p = 0.038$) (Table 4). The correlation coefficient between age and salivary candidal positivity was significant (-0.296 ; $p = 0.022^*$; Table 2).

DISCUSSION

H. pylori as a causative agent for gastritis was first documented by Marshal and Warren in 1983.¹ The bacterium causes progressive damage to the gastric mucosa due to chronic inflammation, and over a period of time, it leads to gastric adenocarcinoma or gastric mucosa-associated lymphoid tissue lymphoma.²⁰ Chronic *H. pylori* infection affects approximately two-thirds of the population worldwide and has a prevalence of approximately 90% in developing countries. Low education levels and low socioeconomic status are associated with an increase in the prevalence of *H. pylori* infection.²¹ Epidemiological data shows that the rate at which a population acquires *H. pylori* infections is higher in developing than in developed countries.²² The presence of *H. pylori* in the oral cavity has been reported by various researchers, who have suggested that its presence acts as a primary extragastric reservoir that can contribute to gastric reinfection following eradication therapy as well as have an active role in person-to-person transmission.^{9,10} However, some studies have reported the absence of any evidence for the oral cavity as a major reservoir for *H. pylori*.^{7,23} This could be attributed to the difficulty in sample collection because the bacteria is found in specialized niches in the oral cavity, such as in plaques rather than in saliva. Anand et al.,²⁴ in their review of the literature on the presence of *H. pylori* in the oral cavity, have found that in patients with *H. pylori* infection, the detection of *H. pylori* in the dental plaque is higher than that from the saliva because plaque acts as a biofilm that facilitates the adherence of the bacteria to the solid surfaces.²⁴

Table 1. Age and Sex Distribution between the Study Groups (N = 70)

Age Groups	Group I				Group II				Group III			
	Male		Female		Male		Female		Male		Female	
	N	%	N	%	N	%	N	%	N	%	N	%
≤30	6	26.1	-	-	3	12.5	-	-	3	60	5	100
31–50	13	56.5	5	71.4	10	41.7	4	66.7	2	40	-	-
>50	4	17.4	2	28.6	11	45.8	2	33.3	-	-	-	-
Total	23	76.7	7	23.3	24	80	6	20	5	50	5	50
P value	0.307				0.450				0.444			

Table 2. Relation between Age and Salivary Candidal Status in Patients with Symptomatic Gastritis (N = 60)

Age Group	Candida in the Saliva		OR	95% CI	p value^	p value
	Present	Absent				
<30	6 (66.7%)	3 (33.3%)	7.5	0.98–68.75	0.035*	
30–50 *	12 (37.5%)	20 (62.5%)	2.25	0.52–10.37	0.36	0.064
>50	4 (21.1%)	15 (78.9%)				

Pearson χ^2 between the age and salivary candidal status ($P = 0.064$). CI, Confidence interval; OR, Odds ratio; *=reference

Table 3. Presence of Salivary *H. pylori* and Salivary *Candida* in Various Groups

Salivary <i>H. pylori</i>	Group I		Group II		Group III	
	Candida		Candida		Candida	
	Present	Absent	Present	Absent	Present	Absent
Positive	4	3	0	4	0	0
Negative	10	13	8	18	3	7
	30		30		10	

Table 4. Salivary *Candida* and Salivary *Helicobacter Pylori* in the Oral Cavity

Status of oral <i>Candida</i> and <i>H. Pylori</i>	Symptomatic Gastritis		OR	95% CI	P value
	<i>H. pylori</i> -positive (n = 30)	<i>H. pylori</i> -negative (n = 30)			
Positive	4 (13.3%)	0			
Negative*	26 (86.7%)	30 (100%)	8.22	1.1–61.495	0.038*
	30	30			

CI, Confidence interval; OR, Odds ratio; *= as reference

The most commonly used method for the detection of *H. pylori* in the gastric mucosa is endoscopic biopsy and Giemsa staining; immunohistochemical analysis is also used. Other methods include the RUT, culture, serology, and UBT to diagnose or monitor the presence of *H. pylori*.¹⁸ In our study, we used Giemsa-stained histological section to detect *H. pylori* in the gastric mucosa.

At sites where the bacteria is present in lower levels, such as gastric secretions, saliva, and stools, PCR

techniques show better sensitivity. We used real-time PCR (using *Urease A*) to detect *H. pylori* in the saliva. Moreover, it has been shown that in patients who received systemic drug therapy for the eradication of *H. pylori* in the stomach, oral *H. pylori* was not eliminated. However, in patients who had concurrently used antiplaque mouth rinses alone or in combination with periodontal treatment, considerable reduction in the prevalence of oral *H. pylori* was observed, along with better eradication rates for gastric *H. pylori*.²⁵ In our study, the patients did not use any mouthwash before sample collection.

Candida albicans is the yeast species more often isolated from various sites in healthy adults, children, and hospitalized patients. The oral *Candida* carriage rate in normal healthy children can be as high as 75% of the study group.²⁶ *Candida* species are highly complex group of micro-organisms with a remarkable ability to adapt to environmental stress exposure to antimicrobials and to the host immune system as well as to changes in host physiology.^{27,28} *C. albicans* have been found to colonize various human body surfaces, such as the oral cavity, genitourinary tract, gastrointestinal tract, and skin.^{28,29} Although, yeast and bacteria have coexisted for years, the biological interaction between them remains predominantly unknown. An association between *H. pylori* and *Candida* was first documented by Siavoshi et al.,³⁰ who proposed that the *H. pylori* BLBs were observed within the vacuoles of the *Candida* as an adaptation for invasion, and subsequently, for its persistence and survival. They further proposed that the association between the bacterium and yeast serves to protect *H. pylori* from environmental stress.³⁰

The presence of *Candida* in patients with gastritis, even in low levels, may cause injury to the epithelium due to the action of proteolytic enzymes secreted by the fungal blast conidia or spores.³¹ The coexistence of *Candida* and *H. pylori* in the gastric mucosa of these patients exhibits a synergistic effect in the pathogenesis of the inflammation.³² An intracellular symbiotic relationship between *H. pylori* and *Candida* has been proposed as an important mechanism for the survival of *H. pylori* outside its well-known niche, the human stomach.^{19,21} We observed that patients with symptomatic gastritis aged <30 years old exhibited 7.5 (OR) times ($P = 0.035$) higher chance of having oral *Candida* than those who were aged >50 years old and this association was significant ($P = 0.064$). As the age increased, the mean CFU/mL increased in the overall study group. This finding could be attributed to the fact that older individuals have other local and systemic factors that could influence and provide a suitable environment for the growth of *Candida*.³³ Moreover, in our study, the prevalence of *H. pylori* infection was lower in the younger age group (20–39 years) and higher in the older age group (40–59 years).

There was no significant difference in the mean CFU between the groups. Although we did not observe a significant difference in mean CFU/mL between the age groups, we observed that when the data were stratified, the mean CFU/mL increased with age in males and females with symptomatic gastritis.

When we studied the correlation between patients with *Candida* and *H. pylori* in their saliva, we observed that subjects who exhibited both the organisms in the saliva had an 8 times increased chance of having gastritis and *H. pylori* positivity in the gastric mucosa and this correlation was significant ($P = 0.038^*$). This finding is similar to those of Salmanian et al.,¹⁹ who suggested

that a symbiotic relationship exists between *Candida* and *H. pylori* in the oral cavity, which facilitates the *H. pylori* gastric reinfection.¹⁹

Our findings showed that salivary *H. pylori* is an extragastric reservoir and indicated that it has a potential role in primary infection and reinfection in gastritis. The synergistic role of oral *Candida* with *H. pylori* is being increasingly recognized and further longitudinal studies are required to understand and for improving the management of gastritis.

CONCLUSION

This study found the presence of *H. pylori* and *Candida* in the saliva of patients with chronic gastritis, showing extragastric reservoir. *H. pylori* and *Candida* in the oral cavity could potentially influence *H. pylori* recolonization of the gastric mucosa leading to recurrent gastritis.

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CONFLICT OF INTEREST

The authors declare that there were no conflicts of interest related to this case report.

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