Original Research Article

Study on association of dental plaque, poor oral hygiene, and periodontal disease with helicobacter pylori infection

S. Aravind Kumar*

Assistant Professor, Department of Dentistry, Apollo Institute of Medical Sciences and Research, Chittoor, India
*Corresponding author email: **aravindmds@gmail.com**

International Archives of Integrated Medicine, Vol. 8, Issue 4, April, 2021.
Available online at http://iaimjournal.com/
ISSN: 2394-0026 (P) **ISSN: 2394-0034 (O)**
Received on: 21-03-2021 **Accepted on:** 05-04-2021
Source of support: Nil **Conflict of interest:** None declared.
How to cite this article: S. Aravind Kumar. Study on association of dental plaque, poor oral hygiene, and periodontal disease with helicobacter pylori infection. IAIM, 2021; 8(4): 61-68.

Abstract

Background: Peptic ulcer, the most common stomach disease is now well accepted as an infectious disease; and the causative agent H. pylori must be treated with antibiotics. Recently attention has been focused on the importance of dental plaque in harboring H.pylori and its role as a potential reservoir for gastric infection and reinfection. This study was undertaken to determine whether dental plaque harbors H. pylori and to determine the association between oral hygiene and periodontal disease status and H.pylori gastritis.

Aim of the study: To determine the presence of H. pylori in dental plaque of patients with good, fair, and poor oral hygiene and correlate with H. pylori in antral biopsy of the same patients.

Materials and Methods: This study was conducted in Aravind Dental Hospital with private gastroenterology clinic collaboration. 110 patients with dyspeptic symptoms and clinical indications for an upper gastroendoscopy were selected for the study. Oral hygiene status and periodontal disease status were examined and study variables were obtained from the patient's history. Among the 110 patients, 55 patients who had a positive H.pylori serology or positive rapid urease test or histologic evidence for the presence of H.pylori in antral biopsy specimens were categorized as cases. The remaining 55 patients who were negative for these tests were controls. The presence of H.pylori in dental plaque was detected by the rapid urease test and culture.

Results: It was found that the association of periodontal disease and poor oral hygiene with H.pylori gastritis was not significant. The RUT was positive in 87.3% of cases and 52.7% of controls. But there was no cultural detection of H. pylori in dental plaque.

Conclusion: Hence the presence of H.pylori in dental plaque is inconclusive. Further studies using a larger sample size and specific methods of detection of H.pylori are required to better assess the role of dental plaque as a reservoir for H.pylori and its relationship with H.pylori infection.

Key words

Cemento Enamel Junction, Calculus Index- Simplified, Campylobacter like an organism, Debris Index- Simplified.

Introduction

Helicobacter pylori are one of the most common bacterial infections in humans. Approximately 50% of the world's population is believed to be infected with H.pylori Serological tests have shown that the carriage rate of H.pylori is reported to be 20 – 80% for adults in the developed world and this figure may rise to more than 90% in the developing world [1]. The majority of infected individuals do not develop the clinically apparent disease, but there is now indisputable evidence that 6-20% of infection results in peptic ulceration, and a smaller proportion (less than 1%) are associated with gastric cancer [2]. Infection in a given individual will result either in the peptic ulcer pathway with associated increased acid output or the chronic atrophic gastritis – carcinoma pathway which is associated with hypo or achlorhydria [3]. Although eradication of H.pylori can be achieved with combination therapy of antibiotics, the possibility of recurrence is very high. The reservoir of H. pylori and its mode of transmission is unclear [4]. A fecal-oral, oral-oral, and gastro- oral route of infection has been suggested. Recently researchers have suggested that the primary extra gastric reservoir for H.pylori is the oral cavity [5]. H.pylori has been detected by various methods in dental plaque, which has led to the suggestion that dental plaque may be responsible for the transmission of the bacteria and possibly serve as a source of reinfection after eradication treatment. However some studies have reported no correlation between dental presentation of the microorganism and H.pylori associated gastritis [6]. The hypothesis that oral flora may be a permanent reservoir of viable H.pylori is still inconclusive. Since human infection by this pathogen appears to involve an oral route, it seems biologically plausible that oral health status directly or indirectly influences the process of H.pylori infection or reinfection [7, 8].

Materials and methods

This study was conducted in Aravind Dental Hospital with private gastroenterology clinic collaboration.110 patients with dyspeptic symptoms and clinical indications for an upper gastroendoscopy were selected for the study. Oral hygiene status and periodontal disease status were examined and study variables were obtained from the patient's history. Among the 110 patients, 55 patients who had a positive H.pylori serology or positive rapid urease test or histologic evidence for the presence of H.pylori in antral biopsy specimens were categorized as cases. The remaining 55 patients who were negative for these tests were controls. The presence of H.pylori in dental plaque was detected by the rapid urease test and culture wrote informed consent was obtained from all patients. The details of a complete history and clinical features of the subjects undergoing endoscopy were obtained. Pre-procedure preparations for oesophagogastroendoscopy were performed according to standard methods. Before endoscopy assessment of oral hygiene status, clinical parameters-probing depth and clinical attachment level were measured and plaque samples were obtained for identification of H.pylori by rapid urease test and culture.Biopsies of gastric tissue were collected from the antrum of patients undergoing endoscopy and specimens were used for 1) histopathology study 2) culture 3) rapid urease test 4) Gram and Giemsa staining for identification of H.pylori infection. Venous blood samples were collected from the patients for serological diagnosis of H.pylori infection.

Inclusion criteria

All patients with complaints suggestive of upper gastrointestinal disease –i.e.; dyspepsia and who

were to undergo endoscopy for the same were included in the study.

Exclusion criteria

- Patients with actively bleeding ulcers, Post gastrectomy individuals, Patients with a history of proton pump inhibitor within 2 weeks of endoscopy or antibiotic intake within one month before the study.
- Patients with a history of chronic use of NSAIDs.
- Patients who undergone oral prophylaxis within the past 6 months.
- Patients on mouth rinses.

The subjects were categorized into two groups cases (n = 55) and controls (n = 55).Subjects with clinical symptoms and a positive test for any of the three diagnostic tests (histopathology, or rapid urease test on antral biopsy specimens or serology) were cases. Subjects with clinical symptoms and a negative test for H.pylori serology or negative rapid urease test and histopathology of antral biopsy specimen were controls. A detailed history and informed consent were obtained from all the subjects after explaining the study procedure. The study variables obtained from the patient's history were age, gender, socioeconomic status, handling of animals, smoking (ex-smoker, current smoker, non-smoker), and alcohol consumption (current, past, never).The periodontal evaluation was done by measuring probing depth (PPD) and clinical attachment level (CAL) using mouth mirror and Williams's periodontal probe. Based on AAP classification 1999, 2 patients with periodontal disease – chronic periodontitis – localized (L) or generalized forms (G) were identified. Localized - < 30% of sites involved and CAL more than1mm.Generalized - > 30% of sites involved and CAL more than 1mm.Subjects who were clinically healthy, with probing depth ≤ 3 mm and no clinical attachment loss were considered as healthy (H) subjects. Probing depth is measured from the gingival margin to the base of the pocket using William's periodontal probe. The probe is passed under the gingiva along the circumference of the tooth. The probe inserted is always maintained parallel to the long axis of the tooth. Three measurements are made on the buccal aspect and three on the lingual aspect of each tooth – a total of six sites per tooth. Clinical attachment level is measured from the cementoenamel junction to the base of the pocket using William's periodontal probe. When the gingival margin is located on the anatomic crown the level of attachment is determined by subtracting from the pocket depth, the distance from the gingival margin to the cementoenamel junction. If both are the same, the loss of attachment is zero. When the gingival margin coincides with the CEJ, the loss of attachment equals the pocket depth. When the gingival margin is located apical to CEJ, the loss of attachment is greater than the pocket depth and therefore the distance between CEJ and gingival margin should be added to the pocket depth. Patients were instructed to fast overnight before endoscopy. Before the endoscopic examination of the patients, plaque samples were collected. Dental plaque was removed from the tooth surfaces with a sterile periodontal curette. The sample was dispersed immediately into a vial containing 1ml of urea broth with phenol red indicator to detect urease activity and in another vial containing 0.2% sterile isotonic saline for culture. Endoscopy was carried out with an Olympus fiber optic endoscope by a Gastroenterologist. Before specimen collection, the endoscope with biopsy forceps was rinsed thoroughly in water and soaked in 2% glutaraldehyde (CIDEX) for 20 minutes. The endoscope was thoroughly rinsed with sterile normal saline just before use. From each subject, four biopsy specimens were taken from antral mucosa 2 cm from the pylorus. The specimens were used for rapid urease test, culture, histopathology, Gram and Giemsa staining. The specimens for rapid urease test were inoculated into vials containing urea broth immediately and the specimens for culture were inoculated into vials containing 0.2% sterile isotonic saline. The specimens for histopathological examination were placed in 10% formalin. The specimens for

culture were transported in an icebox to the laboratory and plated onto culture media within one hour of obtaining the specimen. With aseptic precautions, 2.5ml of venous blood was collected from each patient, the serum separated and stored at - 20˚C, till it was used for IgG antibody estimation.

Statistical analysis

The statistical analysis was done using the computer software program SPSS version 12. Mean and Standard Deviation were estimated for different variables in each study group. Normality of the data was tested in each group by using the Kolmogorov Smirnov test. Mean values were compared between two study groups by using either *Student's Independent t-test* or *Mann-Whitney U-Test*. *Pearson's chi-square test* was used to compare the proportions of the two study groups. In the present study, $p<0.05$ was considered as the level of significance.

Results

Table - 1 shows the master chart of cases and controls with the variables, clinical parameters, and tests done in both groups in the study.

Table – 1: Comparison of gender between cases and controls.

Variable	Category	Cases [n=55]		Controls [n=55]		P –value*
		No.	%	No.	%	
Gender	**Male**	16	29.1	16	29.1	**1.00(NS)**
	Female	39	70.9	39	70.9	

Table - 2: Comparison of mean values between cases and controls.

Variable	Group	Mean ± S.D.	P-value*
Age	**Cases**	44.4 ± 15.2	**0.17 (N.S.)**
	Controls	48.2 ± 12.5	
OHI Score	**Cases**	2.05 ± 1.16	**0.75 (N.S.)**
	Controls	1.98 ± 1.07	
ELISA	**Cases**	159 ± 32	**<0.0001(Sig.)**
	Controls	54 ± 36	
SES Score	**Cases**	9.4 ± 4.1	**0.27** (N.S.)**
	Controls	10.0 ± 4.2	

Table – 3: Comparison of OHI score between cases and controls.

Variable	Category	Cases [n=55]		Controls [n=55]		P – value*
		No.	%	No.	%	
OHI-SCORE	**Poor**	11	20.0	10	18.2	**0.85 (N.S.)**
	Fair	25	45.5	28	50.9	
	Good	19	34.5	17	30.9	

Table - 2 shows the comparison of handling of animals between cases and controls. 20 of 55 cases (36.4%) had contact with animals compared to only 4 of 55 controls (7.3%). This difference was found to be significant with a P-value of 0.001 *Student's independent t-test was used to calculate the P-value.** Mann-Whitney U-test was used to calculate the P-value shows the comparison of smoking habit and alcohol consumption between cases and controls. The proportion of current smokers in cases (38.2%) was slightly higher than controls (29.1). However, there was no significant difference in the proportion of current smokers between the two study groups (P = 0.59). The proportion of people currently having the habit

of alcohol consumption in cases (41.8%) was slightly lower than controls (43.6%). However, there was no significant difference between cases and controls (P = 0.67).

Table - 3 shows the comparison of OHI score between cases and controls. Among cases 19 subjects (34.5%) had good oral hygiene, 25(45.5%) had fair and 11 (20.0%) had poor oral hygiene. Among controls 17(30.9%), 28 (50.9%), and 10 (18.2%) had good, fair, and poor oral hygiene respectively. The observed difference in the oral hygiene status between the two groups was not found to be statistically significant (P = 0.85).

Table - 4: Comparison of periodontal disease and pocket depth ≥ 5mm between cases and controls.

Variable	Category	Cases [n=55]		Controls [n=55]		P –value*
		No.	%	No.	%	
Periodontal disease	Healthy	28	50.9	47	85.5	<0.0001(Sig.)
	Periodontitis	27	49.1	8	14.5	
Pocket depth > 5mm / site	No	28	50.9	47	85.5	<0.0001(Sig.)
	Yes	27	49.1	8	14.5	

Table - 5: Comparison of rut (p) and culture (p) between cases and controls.

Variable	Category	Cases [n=55]		Controls [n=55]		P – value*
		No.	%	No.	%	
RUT P	No	7	12.7	26	47.3	<0.0001(Sig.)
	Yes	48	87.3	29	52.7	
Culture P	Negative	55	100.0	55	100.0	-
	Positive	0	0.0	0	0.0	

Table – 6: Comparison of rut (B), culture (B), gram stain and Giemsa stain between cases and controls.

Variable	Category	Cases [n=55]		Controls [n=55]		P – value*
		No.	%	No.	%	
RUT B	No	0	0.0	55	100.0	<0.0001(Sig.)
	Yes	55	100.0	0	0.0	
	Positive	0	0.0	0	0.0	
Culture B	Negative	55	100.0	55	100.0	<0.0001(Sig)
	Positive	2	0.0	0	1.8	
Gram Stain	Negative	15	27.3	55	100.0	<0.0001(Sig.)
	Positive	40	72.7	0	0.0	
Giemsa stain	Negative	5	9.1	55	100.0	<0.0001(Sig.)
	Positive	50	90.9	0	0.0	

Table - 4 shows the comparison of periodontal disease and pocket depth > 5 mm between cases and controls. 27 (49.1%) subjects among cases had periodontal disease with a pocket depth of ≥ 5mm in at least one site compared to only 8 (14.5%) subjects among controls. This difference was found to be statistically significant (P < 0.001).

Table - 5 shows the comparison of RUT (P) and culture (P) between cases and controls. 48 cases (87.3%) had a positive rapid urease test compared to 29 (52.7%) subjects among

controls. This difference was found to be statistically significant with a P-value of <0.001. H.pylori could not be cultured from dental plaque in any of the 55 cases or controls.

Table - 6 shows the comparison of RUT (B), culture (B), Gram, and Giemsa stain between cases and controls. On antral biopsy specimens, all 55 cases (100%) had positive rapid urease test, while 55 controls had negative RUT. 2 cases tested positive on culture, 40 cases (72. 7%) positive on Gram staining, and 50 cases (90.9%) positive on Giemsa staining for H.pylori. Results of histopathological examination of antral biopsy samples revealed 40 cases with chronic active gastritis, 4 cases with features suggestive of malignancy (adenocarcinoma), 2– cases with acute gastritis, 5 cases of atrophic gastritis, and 4 cases of intestinal metaplasia. Among controls, the picture was that of chronic nonactive gastritis or lymphocytic infiltration.

Table - 7: Association of OHI and periodontal disease with rut p in cases.

Variable	Category	RUT P - No [n=7]		RUT P - Yes [n=48]		P – value*
		No.	%	No.	%	
OHI-SCORE	**Poor**	1	14.3	10	20.8	**0.40 (N.S.)**
	Fair	2	28.6	23	47.9	
	Good	4	57.1	15	31.3	
Periodontal disease	**Healthy**	4	57.1	24	50.0	**1.00** (N.S.)**
	Periodontitis	0	0.0	27	53.8	

Table - 7 shows the association of OHI and Periodontal disease with RUT (P) between cases and controls. The results showed that the association of OHI and Periodontal disease with RUT (P) in cases was not significant, but among controls, the association of periodontal disease with RUT (P) was significant (P= 0.005).* Pearson's Chi-square test was used to calculate the P-value.** Fisher's Exact Test (2 –tailed) was used to calculate the P-value.

Discussion

Periodontal disease may be related to several systemic diseases including increased incidence of atherosclerosis, coronary heart disease, stroke, diabetes mellitus, pre-term low birth weight delivery, and respiratory diseases suggested that oral sepsis might play a part in the pathogenesis of gastric ulcers [9]. Dental plaque has been defined as the diverse community of micro-organisms found on the tooth surface as a biofilm embedded in an extracellular matrix of polymers of host and microbial origin Dental plaque typically adheres to supragingival and subgingival tooth surfaces and it will quickly form in the absence of good oral hygiene measures [10]. Chronic periodontitis is the most prevalent form of periodontitis and occurs in response to chronic plaque and calculus accumulation [11]. Patients with actively bleeding ulcers were excluded from the study because the rapid urease test (RUT) lacks sensitivity in Helicobacter pylori diagnosis when peptic ulcer disease presents with bleeding The gender distribution of cases and controls in the present study was similar indicating that there was little influence of these variables on the disease status of the two groups [12]. Lower socioeconomic status and or a low level of education are associated with an increase in the prevalence of H.pylori infection. As the socioeconomic status of individuals and countries has risen, the prevalence in younger generations has declined. However in developing countries, socioeconomic status and sanitary conditions have improved even more slowly, which is thought to account for the continuing high rates of infection in young people [13]. In the present study, although there were more subjects of lower socioeconomic status, there

was no significant difference in the mean SES score between cases and controls. The handling of animals by the subjects in the two groups was also evaluated. The proportion of subjects who had contact with animals among cases (36.4%) is significantly higher than among controls (7.3%), with a P-value of 0.001 and an odds ratio of 6.13 (by multiple logistic regression analysis) 95% CI: 1.70 to 22.14 [14]. The high degree of association between the handling of animals and H.pylori infection in the present study, suggests that animals may play an important role in the transmission of H.pylori infection and that handling of animals may be an important risk factor for developing H.pylori infection [15]. The oral hygiene status of patients was examined using the Simplified Oral Hygiene Index of Greene and Vermilion 1964 in the present study. The mean OHI score in cases is higher than in controls. However, there is no significant difference in OHI score between cases and controls (P = 0.75) In the present study H. pylori was detected in dental plaque by rapid urease test in patients with good and poor oral hygiene among cases and controls [16]. H. pylori may be part of the normal oral microenvironment and belong to a normal bacterial film that is not a pathogenic reservoir of H. pylori for the stomach. However when the host's immunological defense becomes impaired, bacteria's role as commensal is changed and it becomes a pathogen [17]. H.pylori survives in moderate to advanced periodontal pockets because the architecture and the microcosm of these periodontal conditions promote a viable habitat for microaerophilic and anaerobic micro-organisms. Because dental biofilm can provide urea, urease producing bacteria such as H.pylori may have improved viability in this periodontal environment. Correlation analysis was done between oral hygiene status of the patients and presence of H. pylori in dental plaque and thus with gastric infection in cases and controls. It was not found to be statistically significant [18, 19, 20].

Conclusion

Dental plaque cannot however be discounted as a possible alternate site for the organism. The present methods may be inadequate to reliably isolate the organism from this site. A more comprehensive search for the organism in this environment and other ecological niches within the gingival crevices ought to be conducted to elucidate the role of dental plaque as a potential reservoir for H.pylori. If the oral cavity is a reservoir for gastric infection, even in a minority of individuals, the control of dental plaque along with standard periodontal procedures should be recommended for patients with chronic gastritis or peptic ulcer.

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