Identification of Helicobacter Pylori in Dental Plaques

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ABSTRACT:

Helicobacter pylori (H. pylori) were identified in dental plaque, raising the possibility of future gastritis and peptic ulceration. The aim of the present study was the assessment the association of H. pylori of dental plaque and stomach in a more homogenous population and also to determine the diagnostic value of dental plaque for gastric infection. H. pylori in dental plaque were assessed using three methods, rapid urease test, catalase test and culture method. The significance of the oral hygiene status in these individuals was assessed. Thirty eight patients were positive for H. pylori by rapid urease test, twenty nine patients were positive for H. pylori by catalase test and twenty three patients were positive for H. pylori by culture method out of fifty patients.

Key words: H. pylori, Dental plaque, Rapid Urease test, Catalase test, Culture method

INTRODUCTION

Helicobacter pylori (H. pylori), a microaerophilic gram negative spiral bacteria, first isolated from a human gastric biopsy specimen in 1983, is well adapted to life in the hostile acidic environment of the stomach. The association between H. pylori and the increased risk of duodenal ulceration and antral gastritis has been well established. Hence the importance of preventing reinfection by identifying the potential natural reservoirs of H. pylori. The reservoir of H. pylori and its mode of transmission are unclear, a fecal-oral, oral-oral, and in developing countries a water borne route of infection have been suggested. Studies on gastritis reinfection by H. pylori from an oral reservoir has produced conflicting reports as both supragingival and subgingival dental plaque provide an optimal microaerophilic environment required for the survival of H. pylori. H. pylori were identified in dental plaque in 1989. Some researchers have hypothesized that dental plaque might be the reservoir for H. pylori in those patients with associated gastritis and ulceration. As techniques have improved, this bacterium has been frequently isolated in dental plaque, with some reports showing 100% correspondence between H. pylori containing dental plaque and patients with H. pylori associated gastritis and oral ulceration. Various methods have been used to detect H. pylori in dental plaque, suggesting that dental plaque may be responsible for the transmission of the bacteria and...
possibly serve as a source of reinfection after eradication treatment. *H. pylori* have also been isolated from saliva and denture fitting surfaces.

**METHODOLOGY**

**Study population**

Present study was conducted at V. V. Institute of Pharmaceutical Sciences, Gudlavalleru. The study population was comprised of the patients with a complaint of dyspepsia. All of the selected patients were non-smokers. A history of the following confounding factors was ruled out in patients as an important step toward achievement of a more homogenous study population:

- Previous treatment of peptic ulcer
- Cancer
- Antibiotic therapy or the use of oral antibacterial mouth rinse for any reason during the past year
- Surgical and non-surgical periodontal therapy since a year ago
- Signs of active periodontal disease
- Severe dental caries or recent use of proton pump inhibitors and non-steroidal anti-inflammatory drugs.

The last two factors are important since potential interactions of dental and periodontal pathogens with *H. pylori* could act as a source of bias.

**Dental plaque collection**

Dental plaque was removed from the tooth surfaces with a sterile curette. Plaque was collected by an upward scrape against the tooth surface. The sample was dispersed separately in 1 ml of urea broth with phenyl red indicator to detect the urease activity and in 1 ml of normal saline for catalase test and culture in selective media.

**Urease test**

**Medium used for urease test:** Any urea medium, agar or broth

**Indicator used in urease test:** Phenol red

**Colour change:** Original: orange yellow color

**Final color (in positive test):** Bright pink

**Urease test principle:** Urea is a diamide of carbonic acid. It is hydrolyzed with the release of ammonia and carbon dioxide. Many organisms especially those that infect the urinary tract, have a urease enzyme which is able to split urea in the presence of water to release ammonia and carbon dioxide. The ammonia combines with carbon dioxide and water to form ammonium carbonate which turns the medium alkaline, turning the indicator phenol red from its original orange yellow color to bright pink.

**Catalase test**

Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites; H$_2$O$_2$. The catalase enzyme neutralizes the bactericidal effects of hydrogen peroxide and protects them. Anaerobes generally lack the catalase enzyme. Catalase mediates the breakdown of hydrogen peroxide (H$_2$O$_2$) into oxygen and water. To find out if a particular bacterial isolate is able to produce catalase enzyme, small inoculums of bacterial isolate is mixed into hydrogen peroxide solution (3%) and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production. Catalase test can be used as an aid to the identification of Enterobacteriaceae.

**Members of Enterobacteriaceae family are Catalase positive.**

**Procedure of catalase test (Slide Test):**

1. Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop.
2. Place a drop of 3% H$_2$O$_2$ on to the slide and mix.
3. A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling.
4. A negative result is no bubbles or only a few scattered bubbles.
Culture method
Preparation of Nutrient Agar medium:
Composition:
- Yeast extract 0.4g
- Peptone 1.0g
- Sodium chloride 1.0g
- Agar 3.0g
- Distilled water up to 200ml

Procedure:
1. The medium is prepared and autoclaved at 121°C for 30 minutes.
2. Remove the medium and cool the medium and then pour microbial suspension into it.
3. Transfer the medium into the petri dishes in laminar air flow chamber.
4. Incubate the petri plates in incubator at 37°C. After five days of incubation the colonies showing the following morphology - circular, convex, translucent and glistening were presumptively identified as *H. pylori*.

Statistical analysis
One-way ANOVA was employed for calculating the results for rapid urease test (RUT), catalase test and culture method.

RESULTS AND DISCUSSION
*H. pylori* in dental plaque were assessed using three methods. The rapid urease test, catalase test and culture method. The significance of the oral hygiene status in these individuals was assessed. Thirty eight patients were positive for *H. pylori* by RUT (Fig. 1 and 2), twenty nine patients were positive for *H. pylori* by catalase test (Fig. 3) and twenty three patients were positive for *H. pylori* by culture method (Fig. 4) out of fifty patients (Table 1 and Fig. 5).

The *H. pylori* positivity in duodenal and gastric ulcer patients was assessed. In his unquenchable thirst for conquering the disease, man has focused his research at the cellular and molecular levels to understand the disease process better. Even since the discovery of *H. pylori* by Marshall and Warren in 1982, its role in gastric pathophysiology represents a fundamental change in the understanding of peptic ulcer diseases.

Parronnet in 1998 reported that *H. pylorus* was the most common infection in human. The rate of acquisition of *H. pylori* infection was higher in developing than in developed countries. Even within the developed countries, the prevalence varies between ethnic and racial groups and could be due to differences in cultural background, social, and environmental factors. However, the disease only occurs in about 15% of infected persons. The virulence of the strain was the major determinant of who develop the disease. Important virulence factors responsible for the infected strain were thought to include the spiral shape of the bacterium, flagella that allows it to move rapidly, the presence of enzyme urease which buffer gastric acid, presence of adhesive which are site specific gastric epithelium, production of vacuolating cytotoxin, cytotoxin associate gene production A, and finally the ability to stimulate neutrophils to degranulate. The genetic susceptibility of the host also influences the cause of the disease.

![Fig. 1: Identification of *H. pylori* by RUT](image1)

![Fig. 2: Identification of *H. pylori* by RUT](image2)
Fig. 3: Identification of *H. pylori* by catalase test

Fig. 4: Identification of *H. pylori* by culture method

Table 1: Assessment of diagnostic tests in 50 patients

<table>
<thead>
<tr>
<th>Type</th>
<th>RUT</th>
<th>Catalase test</th>
<th>Culture method</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>38  (76%)</td>
<td>29 (58%)</td>
<td>23 (46%)</td>
<td>90 (60)</td>
</tr>
<tr>
<td>Negative</td>
<td>12  (24%)</td>
<td>21 (42%)</td>
<td>27 (54%)</td>
<td>60 (40)</td>
</tr>
<tr>
<td>Total</td>
<td>50 (100%)</td>
<td>50 (100%)</td>
<td>50 (100%)</td>
<td>150 (100%)</td>
</tr>
</tbody>
</table>

Two main mechanisms were suggested by which *H. pylori* may produce gastric inflammation\(^{13}\). The organism may interact with surface epithelial cells, producing either direct cell damage or liberation of epithelial proinflammatory mediators; and *H. pylori* products may gain access to the underlying mucosa, thereby directly stimulating host nonspecific and specific immune responses involving the liberation of variety of cytokines (TNFα, IL, IL-6, IL-7, IL-10, and IL-12). Direct mucosal damage may be due to adherence of the organisms to the gastric epithelium, vacuolating cytotoxin which include vacuole formation in epithelial cells, variety of enzymes like urease, which by producing ammonia not only protect the organisms from gastric acid but also have toxic effects on the mucosa, and bacterial phospholipase that degrade the phospholipids components of gastric mucosal barrier.

*H. pylori* is the first bacterial infection recognized as the human carcinogen (National institute of Health Consensus Development)\(^{14}\). It is also associated with gastric carcinoma and MALT syndrome, asthma, cerebrovascular, and cardiovascular diseases.

*H. pylori* infections are particularly difficult to eradicate. To control the infection there is need to know about the routes of entry and various reservoirs. Various modes of transmission like oro-oral, feco-oral, and spread by water and through food have been implicated.

Dental plaque has been implicated as a possible source and route of transmission of *H. pylori*\(^{15}\). Krajen\(^{16}\) et al, first reported on the presence of *H. pylori* in dental plaque. Subsequently, various studies reported a wide range of isolation. These variations may reflect on the methods used, technical difficulties, microbiotic complexes, geographic distribution, and host response. Dental plaque can be defined as the soft deposits that form the biofilm adhering to the tooth surface or other hand surfaces in the oral cavity including removable and
fixed restorations. The significance of the biofilm environment has been increasingly recognized in recent years because the environment itself may alter the properties of the organisms. The biofilm community is initially formed through bacterial interactions with the tooth and then through physical and physiologic interactions among different species within the microbial mass. Newer microscopic technique reveals that plaque is actually heterogeneous in structure, with clear evidence of open fluid-filled channels running through the plaque mass. These channels may provide for circulation within plaque to facilitate movement of soluble molecules such as nutrients or waste products. This biofilm matrix acts as a barrier. Substances produced by bacteria within the biofilm are retained within the biofilm. The resistance of bacteria to antimicrobial agents is significantly increased in a biofilm environment. This may be related to the limited diffusion of substances into the biofilm matrix, the slow rate of cell growth in the biofilm environment, and possibility to alter properties of bacteria in response to growth on a surface.

In our study, three methods - RUT, catalase test and culture - were used for isolation of Helicobacter pylori from dental plaque. The specificity and sensitivity of RUT was compared with that of culture. By culture, H. pylori were detected in 46% of the patients.

Antimicrobial therapy frequently failed to cure H. pylori infections. This may be due to sanctuary sites where the organisms reside. One such site may be the oral cavity. The mechanism by which H. pylori reaches the oral cavity is unknown. Anne Marie in 1995 reported on the occasional reflex of H. pylori from the gastric reservoir leading to colonization of the oral cavity, which may be true. The parameters for obtaining plaque that most likely contain H. pylori are unknown. Studies showed that H. pylori are not uniformly distributed in the mouth.

Prospective studies are needed to identify the best methods to ensure that H. pylorus is not missed because of improper sampling. The subgingival microbiota in patients with gingival problems provides a significant and persistent Gram-negative bacterial challenge to the host. These organisms and their products have direct access to the circulation via the ulcerated epithelium. Proper maintenance of oral hygiene will reduce this subgingival pathogenic microbiota and it in turn might control the systemic disease. Whether dental plaque represents a common ecological niche for this organism has not been established, but these findings should encourage the systematic investigation of this site and other possible sources, to gain further insight into the epidemiology of H. pylori.

CONCLUSION

H. pylori, a Gram-negative, microaerophilic motile organism which may be present in the oral cavity as a consequence of gastric reflex. Further studies are needed to confirm whether removal of plaque can cause any change in the recurrence rate. Demonstration of oral carriage of H. pylori, transient or permanent, may have immediate applications with recommendations to prevent person-to-person transmission via the oral-oral route.

Eradication of oral H. pylori by local medication or oral hygiene procedures would rely on the precise identifications of its oral ecological niche. Future directions into usage of polymerase chain reaction and serology test in detection of H. pylori will gain further insight into oral H. pylori, and it would offer the potential for the noninvasive test for the infection.

REFERENCES


