Association of *Helicobacter pylori* in oral cancer patients

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Abstract

**Background:** Few studies have focused on the relationship between *Helicobacter pylori* (*H. pylori*) infection and oral diseases. In this study, we explored the correlation between *H. pylori* infection and oral cancer.

**Methods:** A total of 120 patients of which 60 were oral cancer and another 60 were age- and sex-matched healthy control subjects were enrolled in this study. The *H. pylori* immunoglobulin (Ig) G antibodies in serum were detected by enzyme-linked immunosorbent assay (ELISA) method to assess the status of *H. pylori* infection of our study sample.

**Results:** Prevalence of *H. pylori* infection in the patients with oral cancer were statistically analyzed by independent t test and chi square test. *p*-value was statistically significant.

**Conclusion:** Our findings suggested that there is positive association between *H. pylori* and oral cancer.

**Keywords:** Veneers, aesthetics, fluorosis

Introduction

Cancer is the second leading cause of death in the world after cardiovascular disease. Oral and oropharyngeal cancer grouped together, forms the sixth most common cancer in the world. Oral cancer is associated with mutations in genes that regulate cell growth and apoptosis leading to uncontrolled proliferation of tumor cells, which occur due to the exposure to tobacco, alcohol, betel quid, etc. Recently, alarming changes noticed in the presentation of oral cancer in younger age group i.e. those 40 years or younger with a significant percentage of them having no habits is both enigmatic and disconcerting. Scientists have search for the less explored etiological factors. These factors include the infectious agents, inflammation and chronic trauma. Amongst infectious agents decent amount of studies are available on virus and candida serving as potential causes of Oral Cancer. But the least discussed factor is the bacterial cause of oral carcinogenesis. Among the bacterial origin *Helicobacter pylori* is considered as one of the etiological factor for oral cancers. *Helicobacter pylori* (*H. pylori*) is a gram negative microaerophilic bacterium that inhabits various areas of stomach and duodenum. *H. pylori* causes chronic gastritis and has been associated with several serious diseases of the gastrointestinal tract, including duodenal ulcer and gastric cancer. The relation between *H. pylori* and gastric tumor pathogenesis has been well described, being influenced by *H pylori*’s ability to modify host immune response. It is supposed that it could act in the same way in progression of oral and oropharyngeal carcinoma. Although several previous studies have focused on *H. pylori* infection and oral cancer risk, no definite conclusion on the correlation of *H. pylori* infection with OSCC could be drawn. As there are conflicting results about the role of *H. pylori* infection in OSCC, present study was done to determine the association of *H. pylori* in oral cancer patients using ELISA.

**Materials and methods**

**Study population**

The present study was conducted in the department of Oral Medicine and Radiology, Kamineni Institute of Dental Sciences, and in the department of Microbiology, Kamineni institute Of Medical sciences, Narketpally, Nalgonda district and was approved by the “Ethical Committee” of the institution.
This is a cross sectional study having mainly of two groups; study group and control group. The present study was done on 120 patients of which 60 were oral cancer patients who were clinically and histopathologically confirmed and another 60 were age and sex matched controls who reported to the Department of Oral Medicine and Radiology, Kamineni institute of dental sciences, Narketpally, Nalgonda.

Inclusion Criteria
1. Patients above 18 to 75 years of age of both the sexes were included. 
2. Patients who were diagnosed as oral cancer based on clinical examination and confirmed by histopathology report. 
3. Patients with a negative history of gastric disorders even in their first degree relatives.
4. Patients who have not taken NSAIDS, Corticosteroids, antibiotics or antacids during last 6 months.

Exclusion Criteria
1. Individuals who had taken NSAIDS, Corticosteroid, antibiotics or antacids during last 6 months.
2. Individuals with a history or evidence of gastrointestinal disorders and also in their first degree relatives.

Methodology
Blood samples were collected from both patient and control groups, serum was separated by centrifuging at 3000rpm and serum samples were stored at -20°C till ELISA was done. Anti H. pylori IgG was measured in each sample by ELISA method as instructed by manufacturer. (Biotech Anti H. pylori IgG Kit).

In an empty test tube 200μl of dilution liquid is taken and 10μl of serum is added to this test tube. Now serum samples are diluted at 1:20 ratio.
1st well is considered as a blank in which only 100 μl of dilution sample is added.
2nd well as control – in this 100 μl control solution
3rd well as positive – in this well 100 μl of positive control solution is added
4th well as negative – in this well 100 μl of negative control solution is added.

In remaining all wells 1:20 diluted serum sample was taken and incubated at 370c for 20 minutes. In this step if the antibody is present in the serum it will go and bind to the antigen which is present in the antigen coated ELISA wells. Later Wells were washed with ELISA washer (washer 150ml, distilled water150ml for 96 wells) for three times. After this 100μl of conjugate is added to each well and incubated at 370c for 20 min. In this step, conjugate will bind the antigen and antibody. After this Wells were washed with ELISA washer for three times, to wash the conjugate. Then 100μl of TBS substrate is added to wells and incubated at 370c for 10 min. This is done to color the antigen and antibody bond. Then added 100μl of stop solution to all the wells and waited for 2 sec and then placed in ELISA reader. This is done to stop the antigen and antibody reaction. The optical density of the wells were read by ELISA reader at 420-450nm; where the readings were given by the machine.

Calculated the cut off value by the formula given by manufacturer.
Cut off value = optical density (OD) × control factor (CF)
Control factor value is given by the manufacturer as 0.45
Optical density is determined by the value of 2nd well that is control well.
In the study cut off value is
Cut off value = 3.764×0.45 = 1.69.
Final value = Elisa reader value/ cut off value
For Example = 3.764/1.69 = 2.2 - Positive suggests association with H. pylori.

Interpretation
Greater than 1.1 = positive
Less than 0.9 = negative

Results
Statistical analysis was done using independent –t test and chi square test and p-value <0.05 was considered as statistically significant.

Table 1: Showing the Percentage of Males and Females Affected Byoral Cancer in the Study

<table>
<thead>
<tr>
<th>GENDER</th>
<th>No. of patients out of 60</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>45</td>
<td>75%</td>
</tr>
<tr>
<td>Females</td>
<td>15</td>
<td>25%</td>
</tr>
</tbody>
</table>

Table 2: distribution of oral cancer according to site of its Occurrence in the study

<table>
<thead>
<tr>
<th>SITE OF ORAL CANCER</th>
<th>No. of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tongue</td>
<td>16</td>
<td>26.6%</td>
</tr>
<tr>
<td>Buccal mucosa</td>
<td>13</td>
<td>21.6%</td>
</tr>
<tr>
<td>Upper and lower lip</td>
<td>5</td>
<td>8.3%</td>
</tr>
<tr>
<td>Alveolar mucosa and gingiva</td>
<td>16</td>
<td>26.6%</td>
</tr>
<tr>
<td>Floor of the mouth</td>
<td>7</td>
<td>11.7%</td>
</tr>
<tr>
<td>Sinus</td>
<td>1</td>
<td>1.6%</td>
</tr>
<tr>
<td>Palate</td>
<td>2</td>
<td>3.3%</td>
</tr>
</tbody>
</table>
Table 3: Distribution of Oral Cancer According To TNM Staging in the Study

<table>
<thead>
<tr>
<th>Staging</th>
<th>No. of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>7</td>
<td>11.7%</td>
</tr>
<tr>
<td>Stage II</td>
<td>12</td>
<td>20%</td>
</tr>
<tr>
<td>Stage III</td>
<td>32</td>
<td>53.3%</td>
</tr>
<tr>
<td>Stage IV</td>
<td>9</td>
<td>15%</td>
</tr>
</tbody>
</table>

Table 4: Distribution of Oral Cancer According To Histological Grading In the Study

<table>
<thead>
<tr>
<th>Grade</th>
<th>No. of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well differentiated squamous cell carcinoma</td>
<td>11</td>
<td>18.3%</td>
</tr>
<tr>
<td>Moderately differentiated squamous cell carcinoma</td>
<td>47</td>
<td>78.3%</td>
</tr>
<tr>
<td>Poorly differentiated squamous cell carcinoma</td>
<td>02</td>
<td>3.4%</td>
</tr>
</tbody>
</table>

Table 5: Comparison of ELISA reader values between group A (oral cancer) and group B (control) by independent t – test

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>60</td>
<td>1.72</td>
<td>1.31</td>
<td>0.17</td>
<td>7.1486</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Group B</td>
<td>60</td>
<td>0.45</td>
<td>0.39</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The p-value obtained was 0.0001 suggested statistically highly significant.

Graph 1: Bar Graph Showing Comparison of Elisa Reader Values Between Group A and Group B
Results suggested that *H. pylori* is positively associated with oral cancer patients when compared to healthy control group.

**Discussion**

As we all know that Oral cancer is a major problem in the Indian subcontinent where it ranks among the top three types of cancer in the country. The variation in incidence and pattern of the disease can be attributed to the combined effect of ageing of the population, as well as regional differences in the prevalence of disease-specific risk factors. Public health officials, private hospitals, and academic medical centers within India have recognized oral cancer as a grave problem. Efforts to increase the body of literature on the disease aetiology and regional distribution of risk factors have begun gaining momentum.

Traditionally oral cancer has always been associated with tobacco and areca nut chewing habit. An emerging concept is that tumor development and progression is also largely dependent on the cross talk between immune system and tumor cells. Amongst internal agents tumor associated macrophages and fibroblasts, play a pivotal role. Cytokines released by fibroblasts crucially affect the carcinoma cell behavior and the role of chronic inflammation in tumor progression has now widely accepted. Amongst external agents causing infections like fungal agents (candidiasis) and viruses (HPV and EBV), with oral cancer have already been discussed by many researchers. It is the bacterial population (*H. pylori*) in microenvironment, which is now continuously increasing the concern of scientists towards itself. *H. pylori* association has already been established with gastric, pancreatic and hepatocellular cancers.

Association of *H. pylori* is still a grey area of study as no much studies have been done in respect to the oral cancer. Hence this study was done to know the association between *H. pylori* in oral cancer and healthy controls who were non-gastric patients.

*Helicobacter pylori* (*H. pylori*) is a gram negative microaerophilic bacterium that inhabits various areas of stomach and duodenum. *H pylori* has co-existed with humans for thousands of years, however because the scientist believed the stomach was a sterile organ the bacterium was not discovered until 1980. The bacterium had been observed in 1979 by Australian pathologist Berry Marshal, who did further research on it with Australian physician, J Robin Warren, beginning in 1981. For their discovery of *H. pylori* and its role in gastric ulcer formation Marshal and
Pathogenesis of *H. pylori* in Stomach Diseases

Normally the acidic environment of stomach prevents the survival of viruses, bacteria and other micro-organisms. However *H. pylori* have evolved to be uniquely suited to thrive in the harsh stomach environment. The *H. pylori* bacterium secretes urease, a special enzyme that converts urea to ammonia. Ammonia reduces the acidity of stomach, making it more hospitable home for *H. pylori*.16 The ability to survive in the stomach provides *H. pylori* with a useful hiding place. White blood cells that would normally recognize and attack invading bacteria are unable to cross the blood vessels into the stomach lining. Instead the infective white blood cells continue to respond to the site of infection where they die and release nutrients that feed *H. pylori*. 16 Adherence then occurs via interaction between cell-surface glycolipids and adhesions specific to *H. pylori*. There is also a role played by proteins called cecropins, which are produced by *H. pylori* and inhibit the growth of competing organisms. Once colonization of the gastric mucosa has taken place, the immunogenic properties of *H. pylori* induce an inflammatory reaction with neutrophilic gastritis that ultimately results in the clinical manifestations of the infection. *H. pylori* additionally appears to increase the rate of mucosal programmed cell death (also known as apoptosis). *H. pylori* releases several pathogenic proteins that induce cell injury. For example, the CagA protein, produced by cytotoxic-associated gene A (cagA), is a highly immunogenic protein that may be associated with more severe clinical syndromes, such as duodenal ulcer and gastric adenocarcinoma. In addition, protein products of the vacuolating cytotoxin A gene (vacA) and the A gene induced by contact with epithelium (iceA) are known to be associated with mucosal injury.19 The exact pathogenesis of *H. pylori* in oral cancer is not known but thought to be similar to that of pathogenesis in stomach.

*H. pylori* in Other Cancers

*Helicobacter pylori* is the best-characterized environment risk factor for gastric cancer, which has also been suggested to be linked with diseases in other parts of the human. As the mucosa of the upper aerodigestive tract is in continuity with the gastric mucosa, oral cavity may also be a reservoir of *H. pylori* infection in human. Many studies have detected *H. pylori* in oral cavity where inhabited more than 750 bacterial species. 54,55,56 Ozer Erdem GUR, MD et al., in 2013 done a study to find the association of *H. pylori* in the larynx cancer by detecting IgG antibodies against *H. pylori* antigens using ELISA and also detected organism in the tissue specimens microscopically. Results suggested that there is positive association of *H. pylori* in larynx cancer but they were unable to explain its role in pathogenesis.57 Similarly, few studies have postulated that *H. pylori* may play a certain role in the pathogenesis of Oral cancer.1, with this concept this study was taken up to find out the association of *H. pylori* in oral cancer patients.

Protective Role of *H. pylori* in Other Diseases

Although the disease-causing potential of *H. pylori* infection in stomach, lung, atherogenesis and pancreas, some studies may suggested that *H. pylori* also has some beneficial effects to humans, reducing the risks of esophagus cancer, asthma, allergic rhinitis, and diarrheal diseases.11,58 Yu Chen, et al., in 2007 done a study to find the association between *H. pylori*, asthama and allergy. Conclude that childhood acquisition of *H. pylori* is associated with reduced risks of asthma and allergy 59. Concept behind this is still unexplained.

*H. pylori* in Oral Cavity

The significance of *H. pylori* infection in other human parts including the oral cavity is thought to be different from that in stomach.60 As for the oral cavity, although several previous studies have reported similar genotypes of *H. pylori* strains between oral and gastric mucosa based on histopathologic diagnosis or PCR method, the oral cavity still could not be confirmed as a common place for *H. pylori* colonization, and this bacterium may be present as a transient organism because of the unfavorable surviving environment.11 with this point of view this study was designed to exclude the subjects who were giving positive history regarding gastric infections even in their first grade relatives to find whether *H. pylori* in the oral cavity is transient organism or oral cavity is a common reservoir for *H. pylori* or is it a pathogenic organism in oral cavity.

Few studies stated that dental plaque may be a reservoir for *H. pylori*.37,61,62 Amir Eskandari et al., in 2010 done a study to detect *Helicobacter pylori* using PCR in dental plaque of patients with and without gastritis and results suggested that *H. pylori* was scarce in patients with periodontitis and in patients who are without gastritis. There was a significant association between the presence of *H. pylori* in the dental plaque and gastritis.63 Considering the same concept the present study was designed to know the presence of *H. pylori* in the oral cavity of healthy individuals without gastritis and even in their first grade relatives, to get a conclusion whether oral cavity acts as a reservoir for *H. pylori*. There are conflicting reports about the isolation rate of *H. pylori* from dental plaques. Detection rate ranging from 0 to 90% has been reported in different studies.

The wide variations in the prevalence of *H. pylori* in the oral cavity probably originate from methodological differences among studies rather than from true geographical variations. In the present study there was no association of *H. pylori* in healthy controls who are non-gastric patients compared to oral cancer patients with no gastric infections. This suggests that oral cavity may not be a reservoir of *H. pylori*.

*H. pylori* in Other Oral Diseases

Yuko Ogaya et al., in 2015 done a study on detection of *Helicobacter pylori* DNA in inflamed dental pulp specimens from Japanese children and adolescents using PCR. The results obtained showed that *H. pylori* was detected in inflamed pulp but not in saliva specimens, indicating that an infected root canal may be a reservoir for *H. pylori*.64 In the present study sero prevalence of *H. pylori* was done so, this cannot explain whether *H. pylori* is present in saliva or not. In the literature only few studies have reported the association of *H. pylori* with other RAU, most of the studies concluded the positive association of *H. pylori* in RAU65,66 but few recent studies contradict the statement saying that *H. pylori* is not associated with RAU67.
Diagnostic Tests for *H. pylori*

Currently, there are several popular methods for detecting the presence of *H. pylori* infection, each having its own advantages, disadvantages, and limitations. Basically, the tests available for diagnosis can be separated according to whether or not endoscopic biopsy is necessary. Histologic evaluation, culture, polymerase chain reaction (PCR), and rapid urease tests are typically performed on tissue obtained at endoscopy. Alternatively, simple breath tests, serology, and stool assays are sometimes used, and trials investigating PCR amplification of saliva, feces, and dental plaque to detect the presence of *H. pylori* are ongoing.19 Western Blot (WB) test and in-house ELISA with whole-cell antigen tests are the most reliable tests for the diagnosis of *H. pylori* infection in children and adults. Because of cost and technical demands, in-house ELISA might be more suitable for use in developing countries.20 In the present study *H. pylori* was detected in serum samples of study and control group using ELISA, in which anti *H. pylori* IgG antibodies are detected. This technique was opted as it is a non-invasive, easy to perform, less expensive and easily available in markets.

A study by Preeti et al., in 2015 evaluated the prevalence of *Helicobacter pylori* in Potentially Malignant Disorders and Oral Squamous Cell Carcinoma by cultivating method and results suggested that high prevalence of oral colonization by *H. pylori* in premalignant conditions and OSCC patients.1

Study by Anand Dayama et al. 6 in 2011 done a study to evaluate associations between *H pylori* infection and oral cancer using culture and 16sRNA PCR technique for bacterial identification. Result suggest a possible association of *H. pylori* with an increased risk of oral cancer, but the odds is not statistically significant, this may be because of smaller sample size. In the present study there was positive association between *H. pylori* and oral cancer patients when compared to healthy controls and odds ratio was statistically significant. Soussan Irani et al., in 2013 done a study to assess the association between *H. pylori* and oral lesions such as ulcerative/inflammatory lesions, squamous cell carcinoma (SCC) and primary lymphoma by immunohistochemistry staining, results suggests that they were able to detected the coccoid form of *H. pylori*, which might be proof for its long-standing persistence in the oral cavity and might reveal the role of *H. pylori* in the pathogenesis of the oral disorders examined. And concluded that presence of *H. pylori* might be a risk factor for the developing oral lesions, ulcers and cancers.9 Results of the present study is similar to this study in oral cancer patients but can only detect its sero prevalence of *H. pylori* by ELISA.

But the recent studies suggest that there is negative association of *H. pylori* in oral cancer patients. Xue Meng et al., in 201511 done a study to find the correlation between *H. pylori* infection and oral squamous cell carcinoma (OSCC) by PCR, ELISA and immunohistochemistry. A study by Archana A. Gupta et al., in 201648 was done to access the presence of *H. pylori* in patients suffering from oral cancer by PCR in tissue specimen. And the results suggested that there was negative association with the *H. pylori* in cancer. This may be due to the absence of organism in the particular bit of tissue. But in the present study there is high significant association of *H. pylori* in oral cancer patients when compared to healthy individuals by detecting anti *H. pylori* IgG antibodies using ELISA where there is no chance of missing the organisms antigen even they are treated for gastric infections before 6months also.

In conclusion our present study suggests the positive association of *H. pylori* in oral cancer patients when compared to healthy controls. We also realize that there are several limitations in this study. Firstly, the sample is small, especially in the subgroup analysis. So the results of this study could only be considered as a preliminary exploration. Secondly, the pathogenic mechanism of *H. pylori* infection in the development OSCC remains to be further studied.

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