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ORIGINAL RESEARCH

Evaluation of Prevalence of Bacteria *Helicobacter pylori* in Potentially Malignant Disorders and Oral Squamous Cell Carcinoma

[1]Preethi Sharma, [2]Madhuri Gawande, [3]Minal Chaudhary

ABSTRACT

Cancer is a complex disease that is variable in its presentation, development and outcome. The same heterogeneity and variability exist at the cellular and molecular level. Cancer is a multistep process during which cells undergo intense metabolic and behavioral changes, leading them to proliferate in an extreme and early way to escape supervision by the immune system and ultimately invades distant tissues to form metastases. Globally, almost 20% of cancers are related to infectious agents. Several viruses with oncogenic potential stimulate cell proliferation leading to cancer in animals and humans. Viruses, in particular, have been found to play a major role in the process of cancer involving several organs. Bacterial species related with cancer etiology are varied; however, the infections they cause have common characteristics. The association between *Helicobacter pylori* and gastric carcinoma is well established but the association between *H. pylori* and oral squamous cell carcinoma (OSCC) is not evident. Studies have also reported the existence of *H. pylori* in the oral cavity, but whether the oral cavity serves as an extra gastric source for *H. pylori* or carries the organism only transiently is debatable. Hence, with this aim in the mind, we conducted the present study to evaluate the incidence of *H. pylori* in premalignant disorders and OSCC.

Keywords: *Helicobacter pylori*, Oral squamous cell carcinoma, Potentially malignant disorders.

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INTRODUCTION

Oral cancers are related to the use of tobacco and alcohol consumption. Other factors include viral infections, infection with candida, etc. Bacterial species are associated with different cancers. Increasing evidence shows the association of bacteria with oral cancers. *Helicobacter pylori* is a microaerophilic Gram-negative spiral organism first isolated by Marshall and Warren from human gastric mucosa in 1983.

Helicobacter pylori bacteria can undergo coccoid transformation under hostile conditions and participate in the transmission of infection.[2] Specific *H. pylori* characteristics, such as its gene diversity, helical morphology, citric acid cycle and lipopolysaccharide indicate the acclimination of the bacteria to the gastric mucosa. Newer methods, such as genomic comparision, analysis by transcriptomic method provides an accuracy on the molecular genetics of *H. pylori*. Infection can be diagnosed by invasive methods, e.g. culture, rapid urease test (RUT),[2] histology and molecular methods or non-invasive, such as serology, urease breath test (UBT), stool antigen test and other methods.

The present study aims to assess prevalence of bacteria *H. pylori* in potentially malignant disorders and oral squamous cell carcinoma (OSCC).

MATERIALS AND METHODS

Sample

The study included 50 cases of normal individuals, 50 cases of histopathologically diagnosed cases of potentially malignant disorders and 50 cases of oral squamous cell carcinoma selected from the outpatient department (OPD) of the department of oral pathology.

The materials used for the study were saliva collector, petri plates, microaerophilic gas jar, campylobacter supplement, rapid urease broth, incubator, phosphate buffered saline. Unstimulated saliva was collected in a saliva collector after proper rinsing of the oral cavity. About 1 ml of saliva was collected from every subject and control in a sterile saliva collector. Serial dilution of the specimen was carried out. The media was prepared as per the instructions given along with the media from the Himedia Laboratory Private Limited.

After preparation of the media, the diluted specimen was added on each plate containing media using streak culture method. All the plates are then placed in the

[1]Lecturer, [2]Professor and Head, [3]Professor and Dean

[1,2]Department of Oral Pathology, Sharad Pawar Dental College Wardha, Maharashtra, India

[3]Faculty of Dentistry, Sharad Pawar Dental College, Wardha Maharashtra, India

Corresponding Author: Preethi Sharma, Lecturer, Department of Oral Pathology, Sharad Pawar Dental College, Sawangi (M), Wardha, Maharashtra, India, Phone: 9764848712, e-mail: dr.preethisharma @gmail.com

microaerophilic gas jar. The gas jar is then placed inside the incubator at 37°C for 5 to 7 days. After 7 days, the colonies cultured on petri plates was subjected to rapid urease test (Fig. 1) for confirmation of bacteria. A number of colonies of bacteria grown on each plate were counted.

RESULTS

The microbial assessment of saliva in the respective groups was carried out by culturing of microorganism in the selective medium. The quantitative microbial assessment was done. The results were presented with detailed analysis of *H. pylori* counts in individual groups as descriptive statistics and their significant differences in the groups. We evaluated the presence of *H. pylori* in all the groups. The statistical tests used for the analysis of the result was the Chi-square test, Kruskal-Wallis test (Tables 1 and 2) (Graphs 1 and 2).

Table 1 depicts the correlation of number of colonies counted in leukoplakia and oral submucous fibrosis (OSMF). It was found that there was increased incidence of *H. pylori* count in OSMF when compared to leukoplakia.

Table 2 depicts the number of colonies counted in 50 cases of normal, premalignant conditions and OSCC. It was found that there was an increase in the number of *H. pylori* count in OSCC when compared to premalignant conditions and normal subjects.

Fig. 1: Confirmatory biochemical rapid urease test

Table 1: Correlation of number of colonies in patients with leukoplakia and OSMF

Diagnosis	*Number of patients*	*0*	*1 to 2*	*3 to 5*	*>5*	*p-value (with normal)*
Leukoplakia	29 (58%)	20	8	1	0	7.52, p = 0.023, S
OSMF	21 (42%)	8	13	0	0	23.60, p < 0.0001, S
Total	50 (100%)	28	21	1	0	16.94, p = 0.0002, S
χ^2-value	6.21					
p-value	0.044, S, p < 0.05					

S: Significant

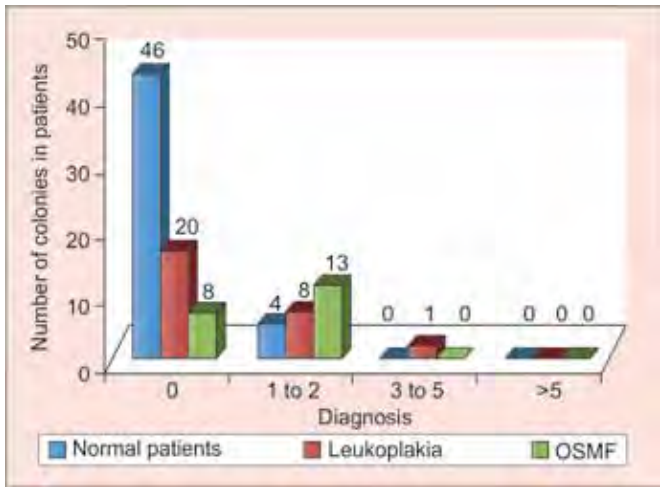
DISCUSSION

In the present study, campylobacter supplement media (Skirrow's) was used to detect *H. pylori*. When the

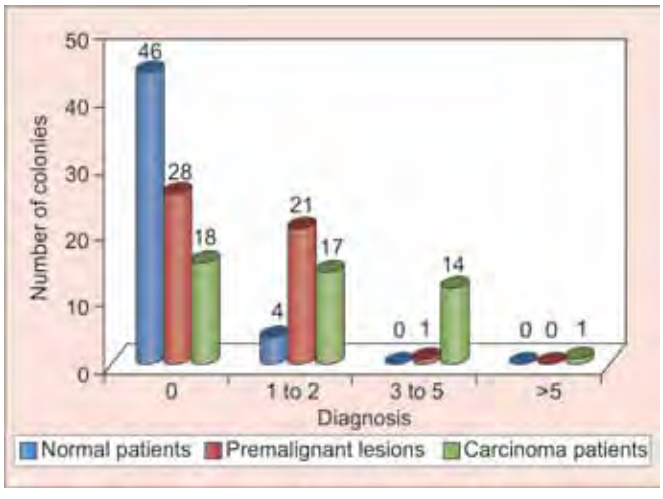
Table 2: Correlation of number of colonies in normal, premalignant and OSCC patients

Colonies counted	*Normal patients*	*Premalignant lesions*	*Carcinoma patients*
0	46	28	18
1 to 2	4	21	17
3 to 5	0	1	14
>5	0	0	1
Total	50	50	50
χ^2-value	—	5.12	61.44
p-value	—	0.023, S	p < 0.0001, S
	Premalignant lesions *vs* carcinoma patients	—	50.82, p < 0.0001, S

S: Significant



Graph 1: Correlation of number of colonies in patients with leukoplakia and OSMF



Graph 2: Correlation of number of colonies in normal, premalignant and OSCC patients

prevalence of *H. pylori* count was compared between leukoplakia and OSMF, it was found that there was increased incidence of *H. pylori* count in OSMF compared to leukoplakia (Figs 2 and 3). The possible risk factors for increased prevalence of the count could be the habit of tobacco and tobacco with lime. The increase in number of colonies in OSMF probably could be attributed to other elements added to tobacco and lime in cases of patients addicted to commercially available tobacco pouches. The oral cavity harbors diversified microflora with more than 750 distinct bacterial taxa that colonise the host tissues. The presence of *H. pylori* in the oral cavity is transient. Bacterial biota becomes pathogenic when their balance is disturbed in the oral cavity. The growth of bacteria is influenced by a variety of factors, such as temperature, pH, availability of nutrients and water, anatomy of oral structures, salivary flow and antimicrobial substances.[3-5] The role of lime could be a source of this disturbance. Lime (calcium oxide in aqueous forms calcium hydroxide) alters the pH in the oral cavity probably over riding with the salivary buffering system and produces an alkaline environment which favors the growth of the bacteria. The presence of $Ca(OH)_2$ in slaked lime also leads to the generation of reactive oxygen species which causes oxidative damage in the buccal mucosa cells of tobacco chewers. The oral cavity is a moist environment which is kept at a relatively constant temperature of 36 to 37°C and pH close to neutrality in most areas, and thus supports the growth of bacteria like *H. pylori*.[4] Oral cancer associated with *H. pylori* infection evolves as a consequence of histological changes in the buccal mucosa due to chronic inflammation, which culminates initially into dysplasia and, at later stages, into cancer.

When the prevalence of *H. pylori* count was compared between normal, premalignant and OSCC patients, it was found that there was increased significance of *H. pylori* count in OSCC when compared to premalignant and normal patients (Fig. 4). This is in accordance with a study by Dayama et al (2011).[6] The results of the study suggested a possible association of *H. pylori* with an increased risk of oral cancer. Tobacco could be the associated risk factor. The oral bacteria demonstrates specific tropism toward different biological surfaces in the oral cavity. This tropism suggests that different bacterial species have different receptors and adhesion molecules that dictates the colonisation of the different oral surfaces. Once the lesion is formed which may be spontaneous or due to underlying changes in the host tissues as a result of external factors, such as tobacco chewing and oral health, specific oral bacteria can colonise and induce inflammation.

The bacteria can lead to chronic infections or produce toxic metabolites that disturb the cell cycle leading to altered cell growth. Chronic infections induce cell transformation and DNA replication through the signaling pathways, such as mitogen-activated protein kinase (MAPK) pathway and cyclin D1, and increases

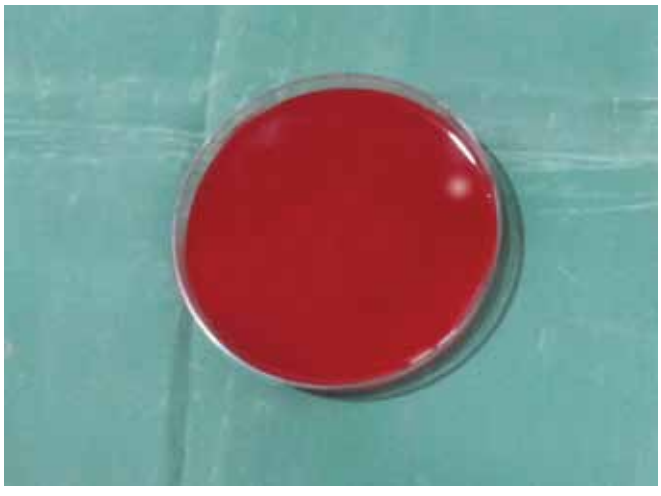


Fig. 2: Small, circular, convex, translucent colony of *H. pylori* on Skirrow's media in leukoplakia

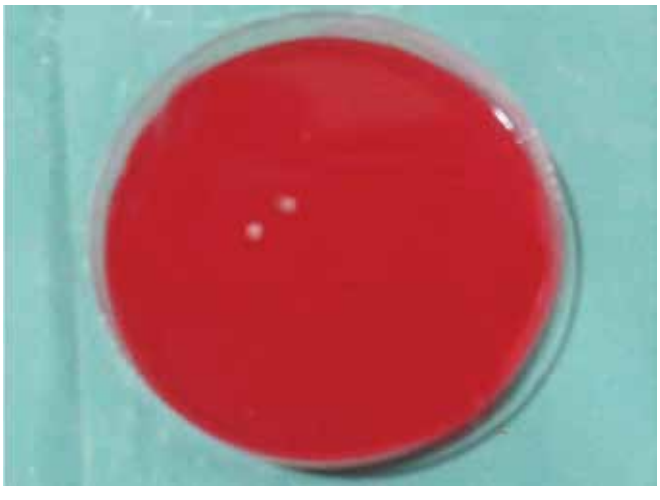


Fig. 3: Small, circular, convex, translucent colony of *H. pylori* on Skirrow's media in OSMF

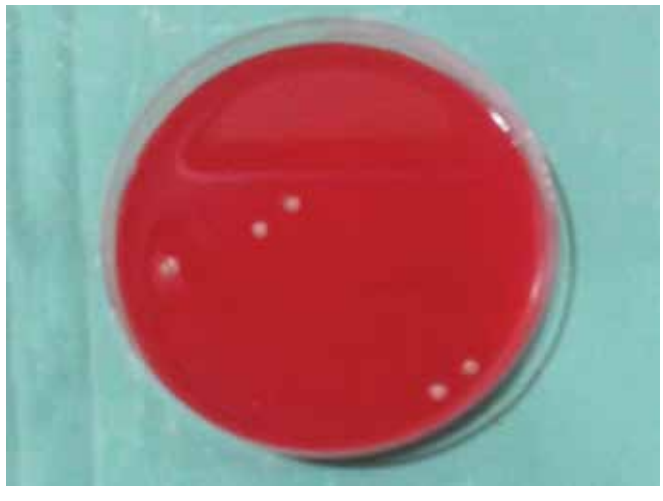


Fig. 4: Small, circular, convex, translucent colony of *H. pylori* on Skirrow's media in OSCC

the rate of cell proliferation and tumor development induced by genetic mutations.[7] Several infections cause intracellular accumulation of the pathogen by modulating the expression of Bcl-2 family proteins leading to suppression of apoptosis or by inactivation of retinoblastoma protein, pRb. This strategy provides an environment in which the intracellular pathogen can survive in spite of the defense by the host immune system to destroy the infected cells by apoptosis. Thus, it allows the partially transformed cells to evade the immune surveillance becoming tumorigenic. The pathogenicity and virulence of *H. pylori* is also determined by a set of genes (denoted as pathogenicity island or PIA), among which the presence of the cytotoxin associated gene cagA codes for protein that confers the pathogenic character to *H. pylori* strains. CagA+ *H. pylori* strains have strong association with cancer.[8] The CagA protein of *H. pylori* has been shown to translocate into the cytoplasm of epithelial cells, where it mediates a number of cellular events including rearrangement of the cytoskeleton, induction of inflammatory mediators through the specific induction of proliferative and oncogenic proteins (via induction of oncogenic transcription factors like nuclear factor κB), activating protein-1, phosphatidylinositol-3-kinase, signal transducer and activator of transcription (STAT)-3 that promote tumorigenic transformation. The prevalence of *H. pylori* infection in precancer and cancer lesions, along with its correlation with habits of tobacco and alcohol use can be considered as a potential risk factors. Thus, *H. pylori* directly and the immune/inflammatory response to *H. pylori* indirectly can influence the rate of epithelial cell proliferation, suggesting this bacterium may be an initiating step in oral carcinogenesis and an important co-carcinogenic factor. To summarise, *H. pylori* infection and chronic inflammation leads to the activation of multiple oncogenic pathways and malignant transformation. The role of inflammation, activation of proto-oncogenes, epigenetic mechanisms, local microenvironment and host genetic susceptibility together determine and promote the progression of cancer.[9,10]

Toxic metabolite products are produced in the *H. pylori* infected mucosa and this is another possible mechanism by which *H. pylori* induces oral carcinogenesis.[11] Among the factors that influence epithelial changes in *H. pylori* infected patients are ROS and RNS. Excessive toxic metabolite production has been reported in human buccal mucosa infected with *H. pylori*, and correlates well with histological mucosal damage and with bacterial load. *Helicobacter pylori* itself has been reported to produce a large amount of superoxide anion ($O_2^{\bullet -}$) in order to inhibit the bactericidal effects of nitric oxide (NO) synthesized by inflammatory cells. On the other hand, $O_2^{\bullet -}$ might be passively produced by electrons leaking from the respiratory mitochondrial pathway of *H. pylori*, since $O_2^{\bullet -}$ production by *H. pylori* can be suppressed by cyanide (CN^-). The cytotoxicity of $O_2^{\bullet -}$ is moderate, but the cytotoxicity of hydroxyl radicals ($^{\bullet}OH$) produced via Fenton's reaction with metals and hydrogen peroxide (H_2O_2) is much higher, and, therefore, *H. pylori* produced $O_2^{\bullet -}$ might indirectly induce epithelial cell injury leading to carcinogenesis.[11]

SUMMARY

Leukoplakia is 'a white patch or plaque that cannot be characterized clinically or pathologically as any other disease'. Oral submucous fibrosis is a chronic insidious disabling condition of the oral mucosa affecting any part of the mouth and rarely the pharynx, larynx and oesophagus. The malignant transformation rate of Leukoplakia is 0.13 to 17.5% and OSMF is 7.6%. Head and neck squamous cell carcinoma are aggressive epithelial malignancies and are among the most commonest neoplasm in the world today. The tumor progression in epithelia has been classified as normal, hyperplastic (non- dysplastic), carcinoma *in situ* and invasive carcinoma.

There are very few scientific studies in the literature on the presence of bacteria *H. pylori* in the oral cavity in cohorts of patients with premalignant conditions and oral squamous cell carcinoma. Hence, we embark on this study to obtain baseline data on *H. pylori* carriage in oral cavity in premalignant conditions and OSCC. For this conventional diagnostic microbial procedures were used and the final identification of the bacteria was done by rapid urease test. The study comprised of 50 diagnosed cases of premalignant conditions, 50 diagnosed cases of OSCC and 50 cases of normal healthy individuals as control group.

The result of the present study can be summarized as follows:

- There was increase in *H. pylori* count in premalignant conditions as compared to normal subjects.
- The *H. pylori* count was increased in OSCC patients as compared to normal healthy individuals and patients with premalignant conditions.

CONCLUSION

The results of the present study confirm the phenomenon of high prevalence of oral colonization by *H. pylori* in premalignant conditions and OSCC patients.

Oral microbiological prevalence studies with frequently monitored, bigger population groups are required to clarify and confirm the present findings and its role in the etiology of OSCC.

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