Original Article

Analysis and Prognostic Significance of *Helicobacter pylori* in Oral Squamous Cell Carcinoma – An Immunohistochemical Study

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Abstract

Background: Oral squamous cell carcinoma (OSCC) cancer is the sixth most common cancer worldwide with approximately 400,000 new cases reported annually. A number of bacterial species are associated with different cancers. The present study aims to analyze the prevalence and prognostic significance of *Helicobacter pylori* in OSCC. **Materials and Methods:** Formalin-fixed paraffin-embedded blocks of previously diagnosed cases of OSCC and normal oral epithelium were retrieved. Corresponding tissue sections were subjected to hematoxylin and eosin staining and immunohistochemical staining. **Results:** The immunohistochemical expression of *H. pylori* scores was higher in oral carcinoma cases than that of normal mucosa which was statistically highly significant ($P = 0.00$). A significant association was observed between immunohistochemical expressions of *H. pylori* scoring and histopathological grades of OSCC ($P = 0.033$) and an inverse correlation was found. **Conclusion:** This study has shown that clinically, *H. pylori* scores were found to be highly expressed in higher stages of OSCC whereas histopathologically, lower grades of OSCC showed higher *H. pylori* scores. *H. pylori* expression is independent of survival rate in these cases in 3-year duration.

Keywords: *Helicobacter pylori*, Immunohistochemical expression, Oral squamous cell carcinoma, Prognosis and survival.

Introduction

Oral squamous cell carcinoma (OSCC) is most common malignancy of head-and-neck region and the sixth most common cancer worldwide with approximately 400,000 new cases reported annually.[1-4] The primary etiological factors include tobacco use, genetic mutations, alcohol, and other possible risk factors include viral and *Candida*, bacterial infections along with poor oral hygiene. In OSCC patients, significantly raised concentrations of certain bacteria in their saliva have been reported. This apparent alteration of the oral microflora could serve as screening aid for malignancy.[5] *Helicobacter pylori*, a flagellated, Gram-negative, spiral, microaerophilic bacteria, is a Class I (definite) human carcinogen as deemed by International Agency for Research on Cancer (World Health Organization) in 1994.[6] It is considered to be the most common chronic bacterial infection among humans.[7] The prevalence of *H. pylori* varies throughout the world and depends largely on the overall standard of living.[8] In developing parts of the world, 80% of the population may be infected by the age of 20, whereas the prevalence is estimated to be 20–50% in industrialized countries, however, these data are inconsistent reported.[9-11]

Mravak-Stipetić *et al*. (1998) detected *H. pylori* in 13.04% of patients with different oral lesions (ulcers, carcinomas, and lymphomas).[12] In another study on head-and-neck malignant and potentially malignant conditions, *H. pylori* were detected in 62.2% of cases.[13] However, there are only

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few studies showing direct evidence of *H. pylori* detection with OSCC s using polymerase chain reaction analysis and immunohistochemistry.[8] Therefore, the present study was aimed to analyze the immunohistochemical expression and prognostic significance of *H. pylori* in OSCC.

Materials and Methods

Formalin-fixed paraffin-embedded blocks of previously diagnosed cases of OSCC (n = 60) and normal oral epithelium (n = 10) were retrieved from the archives of the department of oral and maxillofacial pathology and department of otolaryngology. The cases were divided into following two groups: Group I: Normal oral epithelium or control group, obtained from routine gingivectomy procedure. Group II comprised OSCC cases with a minimum of 1-year follow-up, which was further divided: Group IIa – Well-differentiated OSCC; Group IIb – Moderately differentiated OSCC; and Group IIc – Poorly differentiated OSCC.

From the paraffin embedded blocks, two sections each of 4 µm thickness were obtained using semi-automatic microtome (Shandon Finesse/ME). Corresponding tissue sections were subjected to hematoxylin and eosin staining and immunohistochemical staining using FLEX Polyclonal Rabbit Anti-human *H. pylori*; (Dako) antibody. All slides were assessed for positive or negative histopathological staining. Dark brown spiral or coccoid forms of microorganisms were considered positive for *H. pylori*, observed at ×1000 (×10; eye piece and ×100; nose piece). Histopathological grading was done according to Border's system.

Grading of the density of *H. pylori* colonization (Chen *et al*., 1999):[14]

Score 0 – None, Score 1 – *H. pylori* found only in one place after a careful search Score 2 – Only a few *H. pylori* found, Score 3 – Scattered *H. pylori* found in separate areas/foci, Score 4 – Numerous *H. pylori* in separate areas/foci, and Score 5 – Nearly complete epithelial surface covered by a layer of *H. pylori*.

Statistical analysis of data was done using Chi-square test and Spearman's coefficient of correlation. $P < 0.05$ was considered as statistically significant; while $P < 0.01$ was considered as statistically highly significant.

Results

This study has shown the presence of immunohistochemical expression of *H. pylori* in normal mucosa and also its affiliation with histopathological grading of OSCC. Clinically, *H. pylori* score was found to be highly expressed in higher stages of OSCC (Stages III and IV and nodal metastasis). Prognostically, it was observed that *H. pylori* scores had no association with survival rate in OSCC. However, histopathologically, it was observed that lower grades of OSCC showed higher *H. pylori* scores.

Discussion

Oral cavity serves as source of micro-organisms which might result in infection of stomach and gut or it may serve as transmission gate of external germs for further colonization of GIT. In OSCC, *H. pylori* infection along with tobacco or alcohol adds synergistic effect.[15] The accepted gold standard for the diagnosis of gastric *H. pylori* is the culture technique. However, microbiological culture of *H. pylori* obtained from the oral cavity has been met with limited success.

In the present study, coccoid forms of *H. pylori* were observed in OSCC using IHC which was in accordance with Irani *et al*.[16] and Grimm *et al*.[8] who also observed *H. pylori* in coccoid form in OSCC. The coccoid form of *H. pylori* is viable, more resistant to antibiotics and can spread to infect other cells which lead to its longstanding persistence in the oral cavity and also reveal the role of *H. pylori* in the pathogenesis of oral cancer suggesting an association between the presence of *H. pylori* in the oral cavity and oral cancer.[16] Okuda *et al*. suggested that the coccoid form of *H. pylori* maintains the integrity of the nucleic acid contents and active DNA synthesis. In the present study, a statistically significant immunohistochemical expression of *H. pylori* scores and histopathological grades of OSCC (Chi-square test, $P = 0.033$) were observed (Table 1) which were in accordance with Okuda *et al*.,[17] Dayama *et al*.,[18] Grimm *et al*.,[8] and Amiri *et al*.[19] In our study, it was found that higher the histopathological grade of OSCC, lower the score of *H. pylori* expression. Thus, an inverse correlation was observed (Spearman's correlation coefficient, $r = -0.237$) between *H. pylori* infection and OSCC which was in accordance with Meng *et al*.[20] who also observed an inverse correlation between *H. pylori* infection and OSCC development (Spearman's correlation coefficient $= -0.191$, $P = 0.012$).

TNM staging system is an effective anatomic staging system used to assess the fundamental characteristics of a cancer mass such as the anatomic extent of the primary tumor, involvement of regional lymph nodes, as well as distant metastasis.[21] In

Table 1: Distribution of cases based on *H. pylori* scoring in different histopathological grades of OSCC and normal oral mucosa

Group	Number of samples	*H. pylori* scores in number of samples				
		Score 0	Score 1	Score 2	Score 3	Score 4
I – Normal mucosa	10	1	9	0	0	0
II – OSCC	60	0	0	15	23	22
IIa – WDSCC	20	0	0	3	8	9
IIb – MDSCC	32	0	0	8	12	12
IIc – PDSCC	8	0	0	4	3	1
Total	70	1	9	15	23	22

H. pylori: *Helicobacter pylori,* OSCC: Oral squamous cell carcinoma, WDSCC: Well-differentiated oral squamous cell carcinoma, MDSCC: Moderately differentiated oral squamous cell carcinoma, PDSCC: Poorly differentiated oral squamous cell carcinoma

Table 2: Correlation between *H. pylori* scores and TNM staging of oral squamous cell carcinoma

H. pylori	Number of samples	T stage				N stage					M stage		Clinical staging			
		T1	T2a	T2b	T3	N0	N1	N2a	N2b	N3	M0	M1	I	II	III	IV
Score 0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Score 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Score 2	15	2	12	1	0	5	6	3	1	0	14	1	2	3	6	4
Score 3	23	5	11	7	0	7	9	4	2	1	22	1	4	4	8	7
Score 4	22	6	11	1	4	9	4	1	2	6	22	0	4	5	4	9
Total	60	13	34	9	4	21	19	8	5	7	58	2	10	12	18	20

H. pylori: *Helicobacter pylori*

the present study, no statistically significant association was observed (Table 2) between *H. pylori* scores and T stage (Chi-square test, $P = 0.438$ and Spearman's correlation coefficient, r = 0.026) which is in accordance with Grimm *et al*.[8] About 21% of cases were in Stage T1, 71% of cases were in Stage T2, and 8% of cases were in Stage T3. In contrast, Burduk[22] observed a positive detection of cagA gene for *H. pylori* ranging from 2.1% to 8.6% for T2, from 65.7% to 68.6% for T3, and from 22.8% to 25.7% for T4 stages, that is, the majority of cases belonged to Stage T3 but was found to be statistically not significant (T3 vs. T4, $P < 0.9010$). In our study, no statistically significant association was observed between *H. pylori* scores and N stage (Table 2) (Chi-square test, $P = 0.170$ and Spearman's correlation coefficient, r = 0.082), which is similar to Grimm *et al*.[8] In the present study, 65% of cases with positive *H. pylori* showed lymph node involvement. Similar results were shown by Bruduk[22] who recorded an increase of cagA gene presence in LSCC in patients with positive cervical lymph nodes (N+) 77.1% of supraglottic region and 75.6% of subglottic region but were not statistically significant ($P < 0.4071$).

No statistically significant association was observed between *H. pylori* scores and M stage (Chi-square test, $P = 0.256$ and Spearman's correlation coefficient, r = −0.149) of OSCC in our study. So far, no study has been reported showing correlation between *H. pylori* scores and M stage. Furthermore, no statistically significant association was observed between *H. pylori* scores and clinical stage of oral cancer (Chi-square test, $P = 0.497$ and Spearman's correlation coefficient, r = 0.26) (Table 2). Most of the cases with OSCC and positive *H. pylori* expression were at clinical Stages IV (33%) and III (30%) and fewer were at clinical Stages II (20%) and I (17%) which were in accordance with Bruduk.[22] Thus, cagA gene associated with *H. pylori* was more frequently observed in higher clinical stages of LSCC (T3 and T4 vs. T2) and with nodal metastasis but was not statistically significant ($P < 0.8015$). In contrast to our study, Grimm *et al*.[8] found highly significant association between *H. pylori* and clinical stage of OSCC ($P < 0.05$).

In the present study, no significant association was found between *H. pylori* scores and 3-year survival rate of patients with OSCC, whereas Burduk[22] observed a lower survival rate among patients with the positive cagA gene expression in LSCC. In the present study, patients with recurrence of the disease and positive *H. pylori* scores had worse prognosis which in accordance with Burduk[22] (Table 3).

Table 3: Prognosis with respect to *H. pylori* scoring in oral squamous cell carcinoma

H. pylori	Number of samples	Prognosis		
		No recurrence	Recurrence	Death
Score 0	0	0	0	0
Score 1	0	0	0	0
Score 2	15	6	6	3
Score 3	23	8	5	10
Score 4	22	6	6	10
Total	60	20	17	23

H. pylori: *Helicobacter pylori*

Conclusion

H. pylori are an uncommon pathogen in oral tissue. However, there is less evidence of its involvement in OSCC. Within the limitations of our study, we conclude that *H. pylori* expression does not affect prognosis in OSCC patients or *H. pylori* expression is independent of survival rate in these cases in 3-year duration. Thus, larger sample size studies might add to a clearer picture and long-term follow-up is required for prognostic confirmation.

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