

Perineural invasion in oral squamous cell carcinoma and their correlation with other prognostic markers

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Background: Oral cancer is the eighth most common cancer in the world, making for 3% of all neoplasms. About 95% of oral malignancies are oral squamous cell carcinomas (OSCC). It has a negative impact on survival rates and is linked to severe morbidity and recurrence. Perineural invasion is one clinicopathologic feature that has an impact on its prognosis (PNI). It is the third most typical way that neurotropic tumors spread and is associated with aggressive behavior. **Objectives:** In this study, our aim was to assess the presence of PNI on routine examination and on IHC(S-100) in cases of OSCC correlating it with tumor size, lymph node status, tumor grades, and lymphovascular invasion (LVI). **Materials and Methods:** This study was conducted on 60 histopathologically proven cases of different histopathological grades and variants of OSCC. The tissue sections were examined for PNI and were further compared with tumor size, grading, lymph node status, and LVI. **Results:** A Chi-square test was performed. A percentage positivity of 38.3% (23 cases) among 60 cases was found with 100% of concordance on both routine examination and IHC. PNI positivity of 44.4% and 42.8% was observed in T3 and T4 tumor stages with positive correlation. PNI was found in 31.4% and 66.66% of OSCCs with moderate and good differentiation, respectively. The fact that the difference was not statistically significant highlights the fact that PNI can be present in well-differentiated tumors regardless of tumor grade. Regarding the state of the lymph nodes, PNI positive is not important. With a highly significant value, a positive association between PNI and LVI is discovered. **Conclusion:** According to our study, PNI incidence in OSCC reached 38%. LVI and tumor thickness have strong relationships with PNI. Every surgical specimen with OSCC should therefore be examined for the presence of PNI because it has a significant predictive value and affects treatment choices, recurrence, and distant metastases.

KEY WORDS: Lymphovascular invasion, oral squamous cell carcinoma, perineural invasion

INTRODUCTION

Oral cancer is most common malignant neoplasm in the world wide. It accounts for approximately 2% of all cancers and 1%

of all cancer deaths. It is a global health problem with rising incidence and mortality. Every year more than 3,00,000 new cases of oral cancer are diagnosed and about 95% of them are squamous cell carcinoma of oral cavity.^[1] In India, oral cancer represents a leading problem constituting up to 42% in males and 18% in females.^[2] Common locations include the lip, tongue, buccal mucosa, labial mucosa, floor of the mouth, gingiva, hard palate, and soft palate. Oral squamous cell carcinomas (OSCCs) are the most common oral malignancy with a poor 5-year survival rate. It is estimated that more of 90% of all oral neoplasms are squamous cell carcinoma.^[3] Gender, age, and race have all been

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kindred with differences in OSCC incidence, mortality, site, grade, histological type, and tumor stage at diagnosis. Squamous cell carcinoma of oral cavity has a multifactorial etiology. The cause of oral cancer in the western world is the use of tobacco and alcohol.^[4] Malignant cells' primary characteristic is their capacity to separate and infiltrate nearby structures as well as remote locations. Increased lymphatic and hematogenous spread to distant areas is the cause of increased loco regional recurrence and metastasis. Perineural invasion (PNI) is an alternate form of tumor dissemination with limited study evaluation.^[5] PNI is a type of tumor spread exhibited by malignancies which are neurotropic in nature and corresponds with its aggressive pattern, disease recurrence, and mortality. In head-and-neck incidence of PNI is as high as 80%. PNI in OSCC is a significant predictor of outcome. PNI is tropism of cells of tumor for nerve bundles in the adjoining stroma. PNI has been defined in a number of different ways. PNI was broadly defined by Batsakis as the invasion of tumor cells into, around, and through the nervous system. Liebig *et al.* added "tumor in close contiguity to nerve and involves at least 33% of its circumference or tumor cells within one of the three layers of the nerve sheath" to the earlier criteria in addition to other changes. PNI is considered present when tumor cells are found in the epineurium or perineural space. Tumor cells not going in to the perineural space, but present nearby to the nerve are not taken as PNI.^[6] Immunohistochemical stains can be helpful in defining the extent of PNI, demonstrating the presence of tumor in and around nerve in subtle cases. One of the most useful stain is S-100, a group having low molecular weight acidic proteins enriched in neural tissue with calcium bound region. S 100 is a family of calcium binding proteins comprises 19 members that are differentially expressed in large number of cell types. S-100 is found in glial cells of the central and peripheral nervous system, in melanocytes, chondrocytes, and adipocytes. This antibody is intended for use to qualitatively identify specific antigen by light microscopy.^[7]

MATERIALS AND METHODS

A cross-sectional type of observational study was carried out in the department of pathology of a tertiary medical institute for the duration of 12 months. The clinical findings and other related information were obtained from the patients aided by the requisition forms of the biopsies received. The Specimens were received, grossed, processed, and stained for evaluation. Microscopic assessment of grade, LVI, PNI, and lymph node status along with p TNM stage using AJCC 8th edition, 2018, was taken into account. Thereafter, formalin fixed paraffin embedded tissue blocks were taken up for IHC according to the manufacturer's recommended protocol. S 100 IHC staining was done by primary antibody S 100 Beta (EP32).

Statistical Analysis

- Coding, entry of the data, its clearing, and compiling will be done in excel sheets.
- The data will be imported in Statistical Package for the social Sciences version 23.0 where means and standard deviations will be calculated.

- Depending on the distribution and type of data, quantitative data, expressed in means will be analyzed by *t*-test and proportions through Chi-square test.
- $P < 0.05$ will be considered statistically significant.

OBSERVATION AND RESULTS

The demographic data are illustrated in Table 1.

The most common site of oral cancer 22(36.7%) was found to be buccal mucosa and tongue. Habit history of paan and gutka chewing, cigarette smoking, and areca nut chewing was noticed in majority of the patients of OSCC. Twenty-three out of the 60 patients showed PNI and 37 patients showed no evidence of PNI histopathologically and on immunohistochemistry with 100% of concordance [Figure 1]. The relationships between PNI and clinicopathologic factors have been summarized later.

All cases (60) of OSCC were evaluated for the size of the tumor (T), with two cases belonging to T1, 21 cases of T2, nine cases of T3 and 28 cases of T4. PNI positivity was seen in 33.33% of T2, 44.44% of T3, and 42.85% of T4. The Chi-square test of these OSCC cases with or without PNI in relation to tumor size showed a non-significant *P* value 0.604 ($P > 0.05$).

Table 1: Demographic data

Characteristics	Total number cases
Gender	
Male	50
Female	10
Site	
Alveolus	07
Ary-epiglottic fold	02
Buccal mucosa	22
Floor of mouth	02
Hard palate	02
Soft palate	03
Tongue	22
Tumor	
T1	02
T2	21
T3	09
T4	28
Lymph node	
No	24
N1	09
N2	10
N3	17
Grade	
Well	47
Moderate	12
Poor	01

In different grades of OSCC, out of 47 cases of well-differentiated squamous cell carcinoma, 31.91% of cases show PNI. Among 12 cases of moderately differentiated squamous cell carcinoma, 66.67% of cases show PNI and in one case of poorly differentiated squamous cell carcinoma, PNI is not found. Thus, it can be concluded that PNI status is independent of the degree of differentiation of tumor. This difference was found to be statistically insignificant at $P = 0.063$.

Among the total of 60 OSCC cases, 36 (60%) cases showed lymph node involvement while 24 (40%) did not. Among the PNI positive cases, maximum (8) were present in pN3 category, that is, multiple ipsilateral/contralateral followed by pN0 (7) cases, that is, uninvolved followed by pN1 (5) cases,

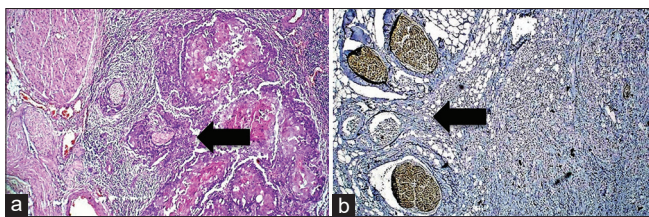


Figure 1: (a) Section showing involvement of nerve by tumor, H&E, $\times 100$. (b) Section showing involvement of nerve by tumor, S 100, $\times 100$

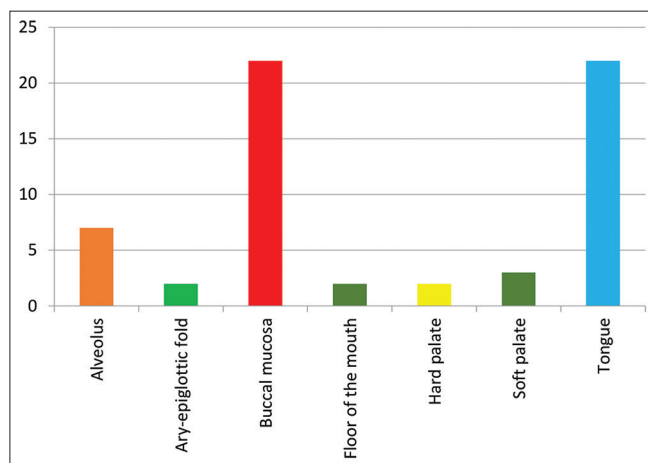


Figure 2: The distribution of data according to site of oral cancer among the study subjects

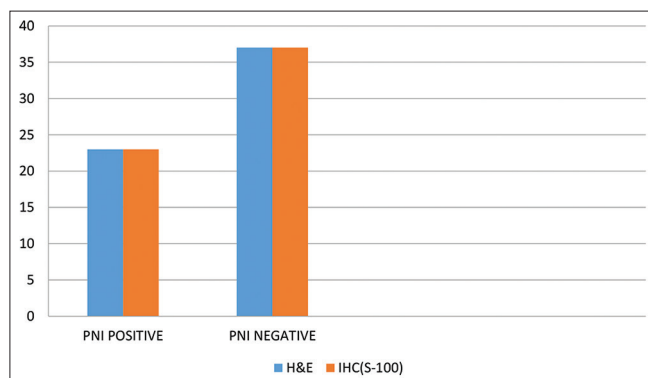


Figure 3: Comparative analysis between routine H&E examination and IHC using antibodies for S-100 in the evaluation of PNI in OSCC

that is, single ipsilateral and pN2 (3) cases, that is, multiple ipsilateral while in PNI negative cases (37), seven cases were with no lymph node involvement (pN0) followed by nine cases with pN3 lymph node status and seven cases with pN2 lymph node status and there were minimum four cases of pN1 were found. The Chi-square test of these OSCC cases with or without PNI in relation to lymph node status showed a non-significant $P = 0.420$.

Among the total of 60 OSCC cases, we studied the correlation between perineural invasion and lymphovascular invasion (LVI) status in which we found that out of total PNI positive cases (23), 10 cases were present with LVI and 13 cases were found to be LVI negative. The Chi-square test showed a significant $P = 0.004$ indicating a strong correlation between the two variables shown in Table 2.

DISCUSSION

OSCC is the sixth most common cancer of among Indian population and is known for its unpredictable course. Long-term survival of individuals having head-and-neck SCC has not improved considerably despite breakthroughs in diagnosis and therapy. Perineural invasion is one clinicopathologic feature that has an impact on its prognosis (PNI). The neurotropic malignancies that are associated with aggressive behavior, illness recurrence, and greater morbidity and mortality display it as the third most frequent kind of tumor spread.

In our study, distribution of patients according to gender was done. It is commonly observed that OSCC is a malignant neoplasm mostly found in males with older age group with higher mortality rates and worst PNI. In the present study, majority of patients were males (83.3%), this was concordant with studies done by Elaiwy *et al.*^[8] in which 91.6% of study subjects were male and 8.6% were females with male to female ratio 10.9:1, our study was also similar to the study of Feller and Lemmer,^[9] Singhi and Westra,^[10] Heck *et al.*^[11]

In our study, patients were also analyzed according to the involvement of site. In our study, it was found that in 60 diagnosed cases of OSCC buccal mucosa being the most common site of involvement (36.7%), followed by lateral border of tongue (36.3%) and alveolus being the third most common site (11.7%) [Figure 2]. This study was similar to Jayasooriya *et al.* in which out of 896 cases of OSCC, buccal mucosa being the most common site in 43% cases which clinically presented as ulcerative growth over mucosa.^[12] This study was dissimilar

Table 2: Distribution of data based on PNI positive and LVI cases

PNI status	LVI		Total	P-value
	Absent	Present		
Negative	33	4	37	0.004
Positive	13	10	23	χ^2 value
Total	46	14	60	8.461

from the study of Pires *et al.* in which out of 346 cases lateral border of tongue (37%) was the most commonest site followed by alveolar mucosa (20%) and floor of mouth (19%).^[13]

In our study, out of 60 cases, 28 patients were in T4 stage and 21 patients were in T2, nine patients in T3 and two patients in T1 stages, respectively. About 42.8% of patients in T4 stage showed PNI histopathologically. Although the percentage positivity of PNI was highest in T3 stage, followed by T4, T2, and T1, sample size was small in these groups to be considered. PNI correlates well with the tumor thickness. This study was similar to Varsha *et al.* in which out of 117 cases of OSCC patients, 71 patients were in T4 stage and 46 patients were in T1, T2, and T3 stages, respectively. About 40.8% of patients in T4 stage showed PNI histopathologically.^[14] The percentage positivity in there study was highest in T2 stage, followed by T4, T3, and T1. Similar results were seen in Deepthi *et al.* in which there was a significantly significant difference in the correlation between tumor stage and PNI, with T3 and T4 displaying the highest levels of PNI positivity.^[5] This brief association sheds light on the relationship between PNI and an increase in tumor size.

In our study, out of 60 subjects, maximum number of PNI positive cases were found to have lymph node pathological stage pN3 followed by pN1 and pN0 and minimum number of cases was found to be of pN2. Correlating PNI with lymph node pathological stage, significant correlation was not found, demonstrating that PNI is independent of the condition of the lymph nodes. These results are consistent with Wallwork *et al.* who also detected no significant association between PNI and nodal status.^[15] Similar results were seen in the study of Deepthi *et al.* Only 30 (42.9%) of the 148 OSCC cases showed PNI, out of the 70 cases that indicated lymph node involvement. Regarding the lymph node status of these OSCC cases with or without PNI, the Chi square test showed that there was no statistically significant correlation between the lymph node status and PNI participation.^[5] Varsha *et al.* investigation's, however, discovered a strong correlation between PNI and lymph node metastases. They discovered that PNI was observed in 34 out of 69 cases (49.3%) of clinically positive nodes but only in 15 out of 47 instances (31.9%) of clinically negative nodes. Even in nodes that are clinically negative, this type of PNI can signal the need for elective neck dissection.^[14]

We studied association between grades of tumor and PNI status among different grades of OSCC, PNI was present in 31.4% and 66.66% of well and moderately differentiated OSCC, respectively, while we have not found PNI in poorly differentiated OSCC. The difference was not statistically significant which emphasizes the fact that PNI can be present irrespective of grade of tumor, that is, in well-differentiated tumors. Even in well-differentiated OSCC, when the outlook is generally favorable, the development of PNI can be a sign of aggressive clinical behavior. Thus, the presence of PNI is irrespective of the histologic grade. These findings are in concordance with Varsha *et al.* where 41.8% of cases were positive for PNI in well-differentiated carcinoma and 48.10% of cases were PNI positive in moderately differentiated carcinoma

and they found no significant association between the histologic grade and presence of PNI.^[14] Similar results were seen in the study Xu *et al.* where the presence of PNI is not significantly correlated with different histologic grades of differentiation^[16] while results of Deepthi *et al.*, Manjula *et al.* and Yong Fu *et al.* studies were discordant to the present study. They discovered that there is a significant rise in PNI positive between grades of OSCC, from highly differentiated to poorly differentiated OSCC.^[5,17,18]

In our study, we also compared and correlated the presence of PNI and LVI together where we found that out of total PNI positive cases (23), 10 cases were present with LVI and 13 cases were found to be LVI negative. Therefore, a positive correlation is found between PNI and LVI with a highly significant value. These results are in concordance with Xu *et al.*, out of 302 cases, 128 are positive for both LVI and PNI showing a highly significant positive correlation. Similar results are seen in study of Huda Alkhadar *et al.* where 75 cases are positive for PNI in which 26 are also positive for LVI while 49 are negative for LVI, showing a highly significant positive correlation.^[16,19] In the present study, out of 60 cases of OSCC, 23 were positive for PNI on either routine H&E examination or IHC staining showing concordance level of 100% (Figure 3).

Finally, pathologic analysis must include assessment of PNI status in OSCC in accordance with the College of American Pathologists' established reporting guidelines.^[20]

CONCLUSION

In the current situation, many therapy decisions are based on TNM staging, imaging studies, and histological findings. In light of this, the present study demonstrated the correlation and proportionality of PNI, as well as its pattern and morphometric analysis in the absence of it with other prognostic parameters of OSCC such as tumor stage, grade, lymph node status, and LVI, which can affect the PNI and result of the disease. Greater nerve width and closer spacing between the nerves and the tumor are linked to a worse PNI in cases lacking PNI. Every surgical specimen with OSCC should be examined for PNI since it has a considerable prognostic value and affects distant metastasis and recurrence. The addition of adjuvant therapy, more aggressive resection, and concurrent management of neck lymph nodes are all required in the presence of PNI. Therefore, histopathologists should look for PNI in the tissue samples from head-and-neck cancers and report their findings to the doctors for patient follow-up and unwavering treatment. Consequently, PNI might be added to the histopathological reporting proforma, particularly in cases of OSCC.

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