Assessment of morphological parameters associated with neural invasion in oral squamous cell carcinoma

M Manjula¹, Punnya V Angadi¹, N K Priya¹, Seema Hallikerimath², Alka D Kale¹

¹Department of Oral Pathology and Microbiology, KLE Academy of Higher Education and Research (Deemed to be university), KLE VK Institute of Dental Sciences, Belagavi, ²Department of Oral Pathology and Microbiology, College of Dental Sciences, Davanagere, Karnataka, India

Abstract

Aims: Neural invasion (NI) is a form of tumor spread distinct from lymphatic and vascular invasion. It has been correlated with aggressive behavior, disease recurrence and increased morbidity and mortality. Despite the importance of NI as a prognostic indicator, the mechanism and associated factors are poorly understood. Hence, the aim of this study was to determine morphological parameters associated with NI in oral squamous cell carcinoma (OSCC).

Methodology: One hundred and five archival specimens of patients with primary OSCC who underwent surgical excision and radical neck dissection were included in the study. The presence of NI was analyzed in slides and correlated with clinical as well as morphological parameters using univariate and multivariate analysis.

Results: NI was identified in 31 cases (29%) of OSCC. NI was significantly associated with tobacco habit, lymphovascular invasion and positive surgical margins. Multivariate analysis further emphasized these factors to be significant risk factors for NI. Peri-NI and intra-NI significantly associated with the size of the tumor, while the distance of invasion was associated with advanced stage.

Conclusion: NI is associated with multiple morphological parameters and its identification may have a significant impact on the management and prognostic evaluation of OSCC.

Keywords: Histopathologic grading, intra-neural invasion, neural invasion, oral squamous cell carcinoma, perineural invasion, prognosis

INTRODUCTION

Oral squamous cell carcinoma (OSCC) accounts to sixth most frequent malignancy across the world and includes no <90% of all oral malignancies. The disease- and management-related morbidity related to OSCC is relatively high and often associated with elevated recurrence rates and poor disease-free survival outcomes in spite of numerous advances in its management.¹ The occurrence of regional or distant metastases are usually the prime causes attributed to the complexity in managing patients with this disease. Neural invasion (NI) is a further distinct metastatic spread that impacts the ability to establish local control.²³

Neurotropism is an explicit feature seen in OSCC that represents an affinity of the tumor cells to the nerve tissue in the stroma and surrounding environment. This has also been generally reported in several other malignancies,
NI is propagation of tumor cells within, around and in the course of the nerves. The tumor cells traverse along the nerve tracks further away from the primary lesion and are frequently overlooked during surgery contributing to the persistence and protracted clinical course predisposing to the development of delayed metastasis.

NI has been considered as a marker of aggressive behavior and is extensively used among the diverse parameters currently in use as outcome predictors for malignancies. It is considered as an independent predictor of poor outcome and decreased survival in colorectal carcinoma, salivary gland malignancies and also in OSCC. Neural extension of OSCC can be established not only by magnetic resonance imaging and computed tomography but also through a comprehensive histopathologic examination of biopsied tissue. There is a marked variation in the frequency of NI reporting in OSCC, ranging from a low 2% and 30% to a high of 82%. Some studies have reported the identification of NI far from the tumor invasive front, in contrast to the presence of an intact nerve within a growing tumor. There are no studies in the literature focusing on details such as the type or quality of NI in OSCC, particularly as it relates to prognosis and relation with clinicopathologic parameters; although, Gil et al. evaluated NI in a variety of tumors of the paranasal sinuses, which included some squamous cell carcinomas and salivary gland malignancies, and concluded that neither the pattern of invasion nor the presence of tumor cells directly within nerve bundles had any prognostic significance.

The present study aims to evaluate the presence of NI in OSCC, determine the morphological parameters associated with NI in OSCC, assess the patterns of NI as well as morphometrically evaluate the nerve diameters and demonstrate the risk factors for NI in OSCC.

**METHODOLOGY**

After obtaining the Institutional Ethical Clearance, a retrospective study based on the review of 105 archival specimens of patients who had undergone surgical resection of the primary tumor and radical neck dissection for OSCC from the Department of Oral Pathology, KLE VK Institute of Dental Sciences, Belgaum (n = 68) and College of Dental Sciences and Hospital, Davanagere (n = 37) was undertaken.

The clinical parameters, including age, sex, the presence or absence of habits, site and tumor size, were noted down from the biopsy requisition forms. The tumor stages were defined according to the TNM classification of the International Union against Cancer.

**Histopathological examination**

The lesional tissue, surgical margins and the lymph nodes were evaluated using two independent observers to clarify the histological parameters and to examine the presence of NI in the lesion. For all the cases incorporated, the following additional histological features with known prognostic potential were evaluated in the lesional tissue according to Bryne et al., i.e., depth of invasion, histologic grade at the invasive front, type of invasive front, type of stroma, extent of inflammation and the presence of lymphovascular invasion. The lymph nodes were assessed for metastases and the surgical margins were also examined for positivity.

**Evaluation of neural invasion**

NI was assessed as positive when cancer cells were seen in the perineural space, the perineurium of the nerve or within the nerve fascicles. Tumors that were only adjacent to the nerves without entering the perineural space were excluded. The pattern of NI was also analyzed according to the presence of the following parameters: peri-NI, intra-NI and both. The distance of NI was categorized as local invasion and distal invasion. The local invasion was defined as invasion of nerves 1 cm or less from the main tumor, whereas distal invasion was defined as invasion of cancer cells >1 cm along the nerve outside the main tumor. The nerve diameter was morphometrically analyzed in areas where NI was evident with Leica QWin image analysis software.
processing and analysis software. The nerves were outlined following which acquisition and analysis software was used to calculate the diameter. The mean of the diameter of different images for the individual case was calculated and tabulated for comparison [Figure 2].

Statistical analysis
An association between the clinicopathological parameters and the NI was evaluated using Chi-square and Fisher's exact test. Multivariate analysis was performed to assess the independent contribution of the clinicopathologic parameters in the prediction of NI. Fisher’s exact test was also employed to evaluate the association of the pattern and distance of NI with the clinicopathologic parameters. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Clinical parameters
Among 105 patients evaluated in this study, 60 were male whereas 45 patients were female. The mean age was 62.5 years (range: 35–90 years). Majority (84%) of the included patients had habit history of tobacco or areca nut chewing along with smoking observed in few patients ranging in duration from 2 to 48 years. Table 1 presents the demographic and clinical data.

Histological parameters
Table 2 depicts the histological evaluation of the lesional tissue. Among these, the parameter depth of invasion revealed majority of tumors demonstrating invasion below the lamina propria, i.e., Grade 3 (51.4%). The histologic grading or differentiation at the invasive front showed 59 (56.7%) well-differentiated tumors, 39 (37.1%) moderately differentiated and only 7 (6.7%) were categorized as poorly differentiated tumors. The most common type of invasive front observed was Grade 2 (37%), i.e., infiltrative solid cords/bands and strands. Most tumors demonstrated a dense stroma (72%) cases with moderate inflammatory infiltration (64 cases). Lymphovascular invasion was evident in 26 tumors (24.7%) with positive surgical margin in 23 cases (21.9%). Histological examination of all levels of lymph nodes of all cases showed the presence of lymph node metastases in 35 cases (33.3%).

Neural invasion and its correlation with clinicopathologic parameters
Out of 105 cases examined, 31 cases (29.5%) showed evidence of NI. Tables 1 and 2 demonstrate the association of NI with various clinicopathologic parameters. The NI was significantly associated with tobacco habit, lymphovascular invasion and positive surgical margins ($P < 0.05$).

The pattern of nerve invasion showed 18 (58.06%) cases with peri-NI, 4 (12.90%) showed intra-NI and around 9 (29.03%) cases showed both intra and peri-NI. The distance of nerve invasion showed local invasion in 17 (54.83%), 12 cases (38.7%) with distal invasion and 2 (6.45%) cases showed both local and distal invasion [Table 3].

Morphometric analysis of nerve in cases which showed NI was also done. The average diameter of nerves ranged from 190 to 731 µm with a mean diameter of $364.6 \pm 158.23$ µm. Majority of the nerves involved with NI were small diameter (<500 um; 77.1%) while 22.6% were >500 µm [Table 3].

There was no statistically significant association between nerve diameter and distance of nerve invasion

Table 1 clinical feature among patients with or without neural invasion

| Variables       | Number of patients (n=105) | Positive - 31 | Negative -74 | $P$   |
|-----------------|----------------------------|---------------|--------------|-------|
| Age             |                            |               |              |       |
| <45             | 28                         | 12 (42.8%)    | 16 (57.1%)   | 0.071 |
| >45             | 77                         | 19 (24.6%)    | 58 (75.3%)   |       |
| Sex             |                            |               |              |       |
| M               | 60                         | 17 (28.3%)    | 43 (71.7%)   | 0.257 |
| F               | 45                         | 14 (31.1%)    | 31 (68.4%)   |       |
| Habit History   |                            |               |              |       |
| Present         | 84                         | 20 (23.8%)    | 64 (76.1%)   | 0.002*|
| Absent          | 19                         | 11 (13.09%)   | 8 (42.1%)    |       |
| Not available   | 2                          | -             | -            |       |
| Size            |                            |               |              |       |
| <4 cms          | 32                         | 13 (59.45%)   | 19 (40.55%)  | 0.154 |
| >4 cms          | 59                         | 13 (67.8%)    | 46 (32.2%)   |       |
| NA              | 14                         | 5             | 9 (64.3%)    |       |

* $P<0.05$, Significant
with the clinicopathologic parameters. However, a significant association related to the pattern of NI and size of the tumor was seen, i.e., both perineural and intra-neural was more commonly seen in tumors measuring >4 cm. In addition, the distance of NI observed was significantly associated with tumor stage with both local and distal invasion more common in Stage 3 and 4 tumors.

Risk factors for neural invasion in oral squamous cell carcinoma

Table 4 summarizes the risk factors for NI in OSCC. The multivariate analysis identified three factors that were significantly associated with NI in OSCC, i.e., habit history (odds ratio [OR]: 0.088 confidence interval [CI]: 0.021–0.370), the presence of lymphovascular invasion (OR: 19.462 CI: 5.334–72.33) and positive surgical margin (OR: 7.346 CI: 1.887–28.600).

**DISCUSSION**

OSCC is a neurotropic malignancy that is conventionally considered as complex to treat and manage. Evidence suggests that demonstration of NI in OSCC should impact adjuvant treatment decisions and surgical management of this disease.\[^{11}\] Previous studies indicate that the rate of NI...
is determined not only by histologic type but also by the anatomical location of the tumor. Nevertheless, detection of NI also depends on the thoroughness of the examining pathologist.\[13\]

The data from our clinicopathologic study demonstrated evidence of NI in 29% of OSCC evaluated which was similar to other studies which showed frequency of NI ranging between 2% and 30%\[6-8\] to as high as 82%.\[9\]

According to the results of the study, a significant correlation was observed with NI and Habit, positive surgical margins and lymphovascular invasion. Several studies\[15,16\] have reported a positive association of NI with lymph node metastasis, depth of invasion and tumor differentiation. In our study too, increased incidence of NI was observed with deep invasion (80%), poorly differentiated tumors (57.14%) as well as with positive lymph nodes (31.4%); however, this did not reach statistical significance. Thus, these results suggest that the tendency of tumors to spread along nerves may be related to their capability for local invasion and proliferation.

A positive association with habit history was seen and also an association was evidenced with lymphovascular invasion which was in contrast with that reported by Rahima \[al\]. This suggests that NI may herald imminent progression to regional and distant metastasis in these patients. In fact, Rahima \[et al\] have shown an association of NI with distant metastasis. They suggested that the tumors with NI should be considered for elective neck dissection based on the justification that metastasis progresses from primary tumors to lymph nodes and then to distant sites.

NI was associated with positive surgical margins suggesting its potential in predicting development of local recurrences and mandating additional adjuvant therapy. Ziv Gil \[et al\]\[11\] found strong association between NI and high rate of positive margin. Based on this, they suggested that patients with NI are 1.4-fold more likely to receive additional radiotherapy after surgery as compared to patients without NI.

The pattern of nerve invasion showed 18 (58.06%) cases with peri-NI, 4 (12.90%) showed intra-NI and around 9 (29.03%) cases showed both intra- and peri-NI. Most data of the patterns of NI of the head-and-neck carcinomas, as well as recent data, suggest that cancer dissemination along nerves occurs exclusively along the peri-neural space and rarely within the nerves’ fascicles. However, Gil \[et al\] found that up to 32% of the cancers of paranasal sinuses showing intra-NI while around 61% showed peri-NI. These enhanced figures as compared to the study may be because they included even salivary gland adenoid cystic carcinomas and sarcomas apart from SCC. The extension of the tumor cells into the peripheral nerves may involve several phases, which include migration, invasion and tumor growth. In addition, the tumor cells can enter the nerve sheath directly invading the perineural space, or they can spread in between the nerve fascicles. Thus, extraneural, perineural and infranuclear invasion may represent diverse stages in tumor propagation triggered by interactions between the tumor cells, nerve tissue and the microenvironment.\[13\]

The distance of nerve invasion demonstrated in 17 cases (54.83%) showed local invasion, whereas only 12 cases (38.7%) showed distal invasion and 2 (6.45%) cases showed both local and distal invasion. Gil \[et al\] also evaluated the distance and found that 63% of SCC of the paranasal sinuses showed local invasion whereas 25% showed distal invasion.\[11\] This finding emphasizes the propensity of the tumor cells to travel along the nerves and present with distal invasion which can be missed during surgery predisposing to local recurrences and poor prognosis. It also underlines the fact NI is a distinct mode of metastases and is an important parameter in prognostication of individual patients.

Further, a significant association was observed with peri-NI and intra-NI and size of the tumor >4 cm and distance of NI was associated with advanced stage (3 and 4). This suggests that larger tumors and advanced stage are more prone to develop NI predisposing to recurrence and additional morbidity. Furthermore, identification of pattern and distance of NI may point to the more aggressive behavior of OSCC.

This is the first study that has analyzed the nerve diameter morphometrically. The mean of all nerves involved with NI was considered for each case and it ranged from 190 to 731 \(\mu\)m with majority of the tumors showing NI involving the small diameter nerves (<500 \(\mu\)m, 77.1%). A study by Maru \[et al\] indicated that PNI diameter measured with an ocular micrometer and defined as the largest focus of PNI in a tumor, was a better predictor of outcome in prostate cancer than PNI status alone. They said that probably tumors with larger foci of PNI are more advanced cancers or there is nerve enlargement within these foci of PNI as result of cancer cell promoted nerve growth phenomenon during PNI.\[7\] Gil \[et al\] also evaluated the nerve diameter as <2.5 mm (38% of SCC) and >2.5 mm (62% of SCC) for NI in cancer of paranasal sinuses.\[11\] However, they did not find any association of the nerve diameter with the clinicopathologic factors similar to our study. The finding
that majority of nerves were of small diameter mandates a detailed review by the pathologist for their identification and reporting.

Multivariate analysis demonstrated that habit, surgical margins and lymphovascular invasion are risk predictors for NI. Thus, this interrelationship has to be studied and evaluated further for prognostication and risk prediction.

The mechanism of NI is still an enigma. It cannot be a pathway of low resistance that acts as conduit for their migration since the several layers of collagen and basement membrane that compose the nerve sheath serve as effective barriers. NI may be associated with reciprocal communication between the tumor cell and nerves as well as the stromal cells that leads to neurite outgrowth, axonal migration and tumor cell invasion. Nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3 and neurotrophin-4/5 are several of the growth factors that have been associated to be participants in this complex mechanism that predisposes to NI. These neurotoxins may serve as viable therapeutic targets for arresting NI.\textsuperscript{[1,18]}

**CONCLUSION**

NI is an important clinical and histopathological feature that is frequently associated with aggressive disease, local-regional recurrence, distant metastasis and poor prognosis. Our study demonstrated that the frequency of NI in OSCC is 29%. Clinicopathologic parameters associated with NI were habit, lymphovascular invasion and positive surgical margin. The peri-ni was seen in 58.06%, whereas intra-NI was observed in 12.9%. The local invasion was seen in 54.8%, whereas 38.7% showed distal invasion. Both were associated with advanced stage and size of the tumor. NI is an important histopathologic and easily accessible parameter of biologic aggressiveness of OSCC. A thorough evaluation by surgical pathologist is mandated for reporting of NI in every surgical specimen of OSCC as it has significant prognostic outcomes. Identification of NI can serve as a guide in selecting patients for further treatment or in identifying those who require more frequent follow-up examinations. The presence of NI necessitates more aggressive resection, coincident management of the neck lymph nodes, and the addition of adjuvant therapy.

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**Conflicts of interest**

There are no conflicts of interest.

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