

Role of Perineural Invasion as a Prognostic Marker in Oral Squamous Cell Carcinoma: A Cross-sectional Study

R RANJITH KUMAR¹, TN SURESH², K SNEHA³, A SAGAYARAJ⁴



ABSTRACT

Introduction: Oral Squamous Cell Carcinoma (OSCC) accounts for nearly 90% of oral malignancies and is often diagnosed at advanced stages. Perineural Invasion (PNI) is recognised as an important prognostic factor; however, its assessment lacks standardisation.

Aim: To evaluate the prevalence, microanatomical location, and spatial density of PNI using the PNI classification system and evaluate its association with clinicopathological features in OSCC specimens at a tertiary care hospital in South India.

Materials and Methods: A cross-sectional observational study was conducted on 50 histopathologically confirmed primary OSCC cases, including retrospective and prospective specimens collected between January and December 2025 at the Department of Pathology, Sri Devaraj Urs Medical College, Kolar, Karnataka, India. PNI was assessed according to the PNI classification system on routine Haematoxylin and Eosin (H&E) stained sections. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 29.0 (IBM Corp., Armonk, NY, USA). The associations between

PNI and tumour grade or TNM stage tiers were evaluated using Fisher's-exact test, with statistical significance set at $p < 0.05$.

Results: The PNI was identified in 17/50 (34.0%) of cases. All PNI-positive foci were confined to the intratumoural region 17/17 (100.0%), with no extratumoural involvement observed 0/17 (0.0%). Based on spatial density, 10/17 (58.8%) of cases demonstrated low-density PNI (1–3 involved nerves/section), while 7/17 (41.2%) showed high-density PNI (>3 involved nerves/section). PNI was not significantly associated with gender ($p = 0.533$) or histopathological grade ($p = 0.136$). PNI showed no significant association with pathological T-stage ($p = 0.351$), but demonstrated a statistically significant association with regional nodal metastasis ($p < 0.001$).

Conclusion: The PNI serves as a significant indicator of aggressive tumour behaviour in primary OSCC, showing a strong association with regional cervical lymph node metastasis and Lymphovascular Invasion (LVI). Standardised qualitative and quantitative reporting using the composite PNI classification system on routine H&E sections improves pathological risk stratification to support individualised postsurgical treatment planning.

Keywords: Head and neck neoplasms, Lymph nodes, Microenvironment, Neoplasm invasiveness, Pathological staging

INTRODUCTION

The OSCC is the most common type of cancer affecting the oral cavity, making up nearly 90% of all diagnosed oral epithelial malignancies worldwide [1]. On a global scale, cancers of the oral cavity and lip create a massive public health burden, resulting in an estimated 389,846 new cases and more than 178,000 deaths every year [2]. This health burden is heavily concentrated in South Asia, and India has one of the highest numbers of oral cancer cases in the world [2]. Recent data shows that India reports around 143,759 new cases each year, which accounts for over one-third of all global cases and 10.2% of all malignancies diagnosed within the country [2]. This high regional burden is directly linked to the widespread cultural habits of chewing smokeless tobacco products (such as *gutkha* and *khaini*) and betel nut, as well as smoking tobacco, which exposes the oral lining to constant irritation from cancer-causing substances [1].

Predicting patient outcomes and deciding on treatments for OSCC have traditionally relied on standard clinical and pathological factors. Histological grade, Tumour-Node-Metastasis (TNM) stage, Depth Of Invasion (DOI), and surgical margin status are well-established factors used to plan patient care after surgery [1]. However, even patients with early-stage or well-differentiated tumours sometimes experience disease spread, showing how unpredictably oral cancer can behave. For this reason, researchers continue to look for better markers of aggressive disease. PNI which occurs when tumour cells surround or grow into nerve structures is an important marker linked to shorter survival times, higher rates of local recurrence, and a greater chance of cancer spreading to regional lymph nodes [2–4].

Biologically, PNI is not just a passive process where tumour cells follow the path of least resistance along nerves. Instead, it is an active process driven by complex interactions within the tumour microenvironment [4]. Schwann cells, the surrounding tissues, and neurotrophic factors {such as Nerve Growth Factor (NGF) and Glial cell line-Derived Neurotrophic Factor (GDNF)} release chemical signals that actively attract tumour cells toward the nerves [4]. This relationship allows cancer cells to travel along nerve routes, which facilitates tumour spread and disease recurrence throughout the surrounding anatomical areas [4,5].

Despite the clear impact of PNI on how aggressively a tumour behaves, there is still a significant research gap in daily hospital reporting. Standard pathology reports usually document PNI as a simple binary variable, meaning it is only recorded as either “present” or “absent.” This approach completely ignores important details like how many nerves are involved or where they are located [6]. To address this issue, Miller ME et al., introduced a detailed composite grading system that looks at both qualitative features (whether the invasion is inside the main tumour mass or past the outer invasive edge) and quantitative features (the density or total number of involved nerves per tissue section) [6]. This composite grading system provides much better risk stratification than a simple binary report, helping doctors identify high-risk patients who might need extra treatments like radiation therapy or more extensive neck surgery [6,7].

At present, clinical studies using this advanced classification system are limited. Very few studies have systematically checked how the

PNI classification system grading relates to standard TNM stages and histological grades [7]. Furthermore, data regarding this grading system in high-incidence Indian populations is scarce [7,8]. More research is needed to understand how the specific habits of tobacco and betel nut use in India affect the way tumour cells invade local nerve pathways.

The rationale for the present study stems from the need to evaluate the multidimensional PNI classification system which accounts for microanatomical localisation and nerve density to provide superior prognostic risk stratification in OSCC, particularly within high-burden South Asian populations where localised data remains scarce.

The study aimed to determine the prevalence, density, and location of PNI in primary OSCC cases using the composite PNI classification system and to evaluate the association between the different PNI grades and standard tumour characteristics, specifically histological grade and TNM stage, to improve patient risk assessment.

MATERIALS AND METHODS

The present cross-sectional study was done in the Department of Pathology, Sri Devaraj Urs Medical College, Kolar, Karnataka, India. Study sample was collected from January 2025 to December 2025. Institutional ethics clearance (IEC no: SDUAHER/R&D/CEC/SDUMC-PG/353/NF/-2025-26) was obtained from the Institutional Ethics Committee for the study protocol.

Inclusion criteria:

- Histopathologically confirmed primary OSCC cases;
- Availability of complete clinical records and representative tumour tissue blocks;
- Specimens derived from primary wide local excisions with neck dissections.

Exclusion criteria:

- Inadequate or highly fragmented tissue samples;
- Biopsy specimens not fixed immediately in standard formalin solutions;
- Patients who received prior induction chemotherapy or neoadjuvant radiotherapy;
- Recurrent or secondary metastatic disease presentations.

Tumours were histopathologically graded according to the World Health Organisation (WHO) Classification of Head and Neck Tumours (5th edition, 2022) [9] based on structural differentiation features. Pathological TNM (pTNM) staging was performed in strict accordance with the guidelines of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 8th edition [10]. To avoid the observational errors associated with macro-gross specimens, the DOI was verified microscopically on standard histopathological sections. Following the AJCC 8th edition rules, DOI was measured from the reconstructed baseline of the adjacent normal epithelial basement membrane to the deepest continuous invasive point of the carcinoma cells using a calibrated ocular micrometer.

PNI definition: Standardised microscopic PNI Assessment was confirmed when individual malignant epithelial cells, clusters of cells or concentric sheets of tumour were seen infiltrating the perineural space, surrounding at least 33% of the nerve circumference or tracking along the epineural sheath. Simple proximity where tumour cells merely abutted a nerve border without infiltrating the architectural space were not scored as positive [11].

All prepared histopathological slides for each case were thoroughly reviewed under light microscopy across multiple magnifications (x10 for initial scanning; and for structural confirmation) to ensure comprehensive sampling. The total number of separate PNI foci fulfilling the above definition was meticulously recorded for each positive case. Immunohistochemistry was not utilised in the present study; the structural assessment of PNI and the tracking of foci were based entirely on routine H&E stained sections.

PNI Classification system: Each positive focus was classified according to the composite PNI classification system to standardise the assessment: $PNI_{Miller} = (PNI_{Density}, PNI_{presence}, PNI_{Location})$. The PNI location was described as the microanatomical depth of involvement in relation to the primary tumour mass as intratumoural (limited within the volume of the primary tumour) or extratumoural (beyond the active, leading invasive front). The quantitative burden of nerve involvement was expressed as PNI density, calculated as the ratio of affected nerve structures to the total number of examined tumour slides. According to the criteria of PNI classification system by Miller ME et al., the density distributions were classified into two types:

- Low-density PNI (1-3 involved nerves/section)
- High-density PNI (>3 involved nerves/section) [6].

The PNI classification system was selected because standard qualitative binary reporting ("present" vs. "absent") fails to account for spatial and microanatomical variations of nerve involvement [12,13]. By subcategorising PNI based on location and spatial nerve density, this framework offers superior prognostic stratification for identifying high-risk patient subsets requiring tailored adjuvant therapy.

Sample size calculation: The sample size for the present study was determined based on the expected prevalence of PNI in OSCC. Drawing from a comparable historical baseline study by Deepthi G et al., which characterised microanatomical patterns and reported an observed PNI prevalence (p) of 31.8% in oral carcinoma cases, the sample size was mathematically estimated [14]. Using the standard single proportion formula, $n = (Z^2 \cdot p \cdot q) / d^2$, with an allowable margin of error (d) set at 13% and a 95% confidence interval ($z=1.96$), the minimum required sample size was calculated to be approximately 49.3 cases. To fulfill this calculated statistical boundary and ensure full internal data integrity, a final sample size of 50 histopathologically confirmed OSCC cases was selected. An attempt was made to achieve an optimal balance between statistical confidence (95%) and practical feasibility within the fixed time constraints and eligible patient volume of a single-centre tertiary health Institution.

Study Procedure

All surgically resected tissue specimens were fixed in 10% neutral buffered formalin for 24 to 48 hours to ensure optimal structural preservation. Following routine automated tissue processing, the specimens were embedded in paraffin blocks. Tissue blocks were sectioned at a uniform thickness of 3-5 μ m and stained with routine H&E dye. Microscopic slide evaluations were performed independently by two senior pathologists. To ensure objective reporting, the pathologists were completely blinded to the patients' clinical data and endpoints. Any inter-observer disagreements regarding the presence or classification of PNI were subsequently resolved via mutual consensus using a multi-headed discussion microscope. For each case included in the study subjects, the clinical and pathological variables systematically collected and analysed were:

- **Demographic and baseline data:** Patient age and sex.
- **Standard clinicopathological factors:** Tumour site, histological differentiation grade (according to the 2022 WHO classification), pTNM staging components (according to the AJCC 8th edition), and microscopic DOI.
- **Perineural Invasion (PNI) variables:** Microscopic presence or absence of PNI, absolute number of individual PNI foci per section, microanatomical location fronts (intratumoural versus peripheral or extratumoural zones), and spatial nerve density scoring boundaries (number of involved nerves per tissue section) evaluated under the composite PNI classification system.

STATISTICAL ANALYSIS

All collected data were compiled into a spreadsheet and analysed using IBM SPSS Statistics software, version 29.0. Descriptive analysis was performed where categorical variables were presented as absolute numbers and percentages, while continuous variables (such as patient age and DOI) were expressed as mean±standard deviation. The normality distribution of continuous variables was evaluated using the Shapiro-Wilk test. For comparative analysis, categorical associations specifically the cross-tabulations of PNI grades against WHO tumour grades and American Joint Committee on Cancer (AJCC). TNM staging components were evaluated using Fisher's-exact test due to small sample counts in individual cell fields. All statistical tests were two-tailed, and a 95% confidence interval was maintained throughout the study. A p-value of less than 0.05 (p <0.05) was considered statistically significant.

RESULTS

Baseline demographics: The baseline demographic profile of the 50 patients evaluated in the present study showed a higher proportion of male individuals. Out of the total study participants, 34 patients (68.0%) were male and 16 patients (32.0%) were female. The overall age of the patients ranged from 38 to 74 years, with a calculated mean age of 56.4±9.2 years. The majority of the study participants fell within the fifth and sixth decades of life [Table/Fig-1].

Demographic parameter	Subcategory	n (%)
Gender	Male	34 (68.0%)
	Female	16 (32.0%)
Age metrics	Mean age±SD	56.4±9.2 years Range: 38-74

[Table/Fig-1]: Baseline demographics (N=50).

Tumour characteristics: Regarding anatomical site distribution within the oral cavity, the lateral border of the tongue was the most common site, accounting for 22 cases (44.0%), followed by the buccal mucosa in 18 cases (36.0%), the lower alveolus in 6 cases (12.0%), and the floor of the mouth in 4 cases (8.0%). Pathological tumour staging performed strictly according to the AJCC 8th edition guidelines revealed that 3 cases (6.0%) were classified as pT1, 14 cases (28.0%) as pT2, 20 cases (40.0%) as pT3, and 13 cases (26.0%) as pT4a. Lymph node involvement was noted in 20 cases (40.0%), resulting in a final pTNM stage grouping of Stage I in 10 cases (20.0%), Stage II in 14 cases (28.0%), Stage III in 15 cases (30.0%), and Stage IVa in 11 cases (22.0%) [Table/Fig-2].

Tumour parameter	Subcategory	n	(%)
Anatomical site	Lateral border of tongue	22	44.0%
	Buccal mucosa	18	36.0%
	Lower alveolus	6	12.0%
	Floor of the mouth	4	8.0%
Pathological T-stage (AJCC 8 th)	pT1 stage	3	6.0%
	pT2 stage	14	28.0%
	pT3 stage	20	40.0%
	pT4a stage	13	26.0%
Final pTNM stage grouping	Stage I	10	20.0%
	Stage II	14	28.0%
	Stage III	15	30.0%
	Stage IVa	11	22.0%

[Table/Fig-2]: Tumour characteristics (N=50).

Histopathological findings: Histopathological grading according to the WHO 5th edition (2022) classification showed that well-differentiated OSCC (Grade 1) was present in 27 cases (54.0%), moderately-differentiated OSCC (Grade 2) in 22 cases (44.0%), and poorly-differentiated OSCC (Grade 3) in one case (2.0%).

The microscopic DOI measured across the entire sample group ranged from 2.1 mm to 14.5 mm with a mean DOI of 6.8±3.4 mm. Surgical margins were free of tumour cells in 43 cases (86.0%), while 7 cases (14.0%) showed close or involved margins [Table/Fig-3].

Histopathological parameter	Subcategory	n (%)
Tumour differentiation (WHO 2022)	Well-differentiated (WDSCC)	27 (54.0%)
	Moderately-differentiated (MDSCC)	22 (44.0%)
	Poorly-differentiated (PDSCC)	1 (2.0%)
Depth of Invasion (DOI)	Mean DOI ±SD	6.8±3.4 mm Range: 2.1-14.5
Surgical margin status	Free margins	43 (86.0%)
	Close/involved margins	7 (14.0%)

[Table/Fig-3]: Histopathological findings (N=50).

PNI prevalence: Based on the diagnostic criteria requiring at least 33% nerve circumference involvement or direct epineural tracking, PNI was identified as positive in 17 out of the 50 studied cases, yielding an overall prevalence of 34.0%. In the remaining 33 cases (66.0%), PNI was confirmed as absent. Among the PNI-positive cases, the total number of distinct microscopic PNI foci discovered per slide ranged from 1 to 5, with a mean focus density of 2.1±1.1. [Table/Fig-4].

Diagnostic feature	Category status	n (%)
Microscopic PNI status	PNI present (positive)	17 (34.0%)
	PNI absent (negative)	33 (66.0%)
Microscopic PNI foci density	Mean Foci ±SD	2.1±1.1 foci/slide Range: 1-5

[Table/Fig-4]: Perineural Invasion (PNI) Prevalence (N=50).

PNI classification: The 17 PNI-positive cases were further analysed and categorised using the composite PNI classification system to evaluate microanatomical location and density. Based on this the following findings are observed:

- Intratumoural PNI (nerves involved within the main tumour mass) was present in 17 cases (100.0% of positive cases).
- Extratumoural PNI (nerves involved beyond the invasive front) was observed in 0 cases (0.0% of positive cases).

Quantitatively, based on nerve involvement per tissue section:

- Low Nerve Density (1 to 3 involved nerves per section) was observed in 10 cases (58.8% of positive cases).
- High Nerve Density (>3 involved nerves per section) was observed in 7 cases (41.2% of positive cases) [Table/Fig-5].

PNI dimension type	Histological subcategory	n	(%)
Anatomical location matrix	Intratumoural fronts	17	100.0%
	Extratumoural fronts	0	0.0%
Quantitative density burden	Low-density PNI (1-3 Nerves)	10	58.8%
	High-density PNI (>3 Nerves)	7	41.2%

[Table/Fig-5]: PNI classification analysis (n=17).

Association analysis: The cross-tabulation of PNI location according to the PNI classification scheme against primary clinicopathological features across the cohort (N=50) is presented in [Table/Fig-6]. Out of 17 PNI-positive cases, 100.0% (n=17) demonstrated intratumoural PNI, with zero cases exhibiting extratumoural PNI. Intratumoural PNI showed a highly significant association with regional cervical lymph node metastasis (pN+) (82.4% vs. 18.2% in PNI-absent cases;

Clinicopathological feature	Total cohort (N=50)	PNI absent (n=33)	Intratumoural PNI 17 (100%)	Extratumoural PNI (n=0)	p-value
Pathological T-stage					
Early stage (pT1/ pT2)	17 (34.0%)	13 (39.4%)	4 (23.5%)	0	0.351
Advanced stage (pT3/pT4a)	33 (66.0%)	20 (60.6%)	13 (76.5%)	0	
Histological grade					
Well-differentiated (G1)	27 (54.0%)	15 (45.5%)	12 (70.6%)	0	0.136
Moderately-/Poorly-differentiated (G2/G3)	23 (46.0%)	18 (54.5%)	5 (29.4%)	0	
Nodal Metastasis (pN)					
pN0 (Negative)	30 (60.0%)	27 (81.8%)	3 (17.6%)	0	<0.001*
pN+ (Positive)	20 (40.0%)	6 (18.2%)	14 (82.4%)	0	
Lymphovascular invasion (LVI)					
Absent	48 (96.0%)	33 (100.0%)	15 (88.2%)	0	0.111
Present	2 (4.0%)	0 (0.0%)	2 (11.8%)	0	
Depth of invasion (DOI)					
Superficial/moderate (≤10 mm)	30 (60.0%)	20 (60.6%)	10 (58.8%)	0	1.000
Deep (>10 mm)	20 (40.0%)	13 (39.4%)	7 (41.2%)	0	

[Table/Fig-6]: Association of PNI location (intratumoural vs extratumoural) with clinicopathological features.

*Statistically significant associations at p <0.05 derived via Fisher's-exact test

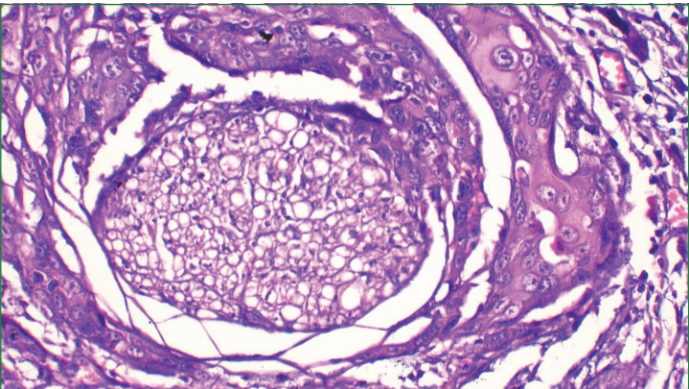
Clinicopathological feature	Total cohort (N=50)	PNI absent (n=33)	Low density (1-3 n/section) (n=10, 58.8%)	High density (>3 n/section) (n=7, 41.2%)	p-value
Pathological T-stage					
Early stage (pT1/pT2)	17 (34.0%)	13 (39.4%)	2 (20.0%)	2 (28.6%)	0.498
Advanced stage (pT3/ pT4a)	33 (66.0%)	20 (60.6%)	8 (80.0%)	5 (71.4%)	
Histological grade					
Well-differentiated (G1)	27 (54.0%)	15 (45.5%)	7 (70.0%)	5 (71.4%)	0.240
Mod/Poor Differentiated (G2/G3)	23 (46.0%)	18 (54.5%)	3 (30.0%)	2 (28.6%)	
Nodal metastasis (pN)					
pN0 (Negative)	30 (60.0%)	27 (81.8%)	2 (20.0%)	1 (14.3%)	<0.001*
pN+ (Positive)	20 (40.0%)	6 (18.2%)	8 (80.0%)	6 (85.7%)	
Lymphovascular Invasion (LVI)					
Absent	48 (96.0%)	33 (100.0%)	8 (80.0%)	7 (100.0%)	0.016*
Present	2 (4.0%)	0 (0.0%)	2 (20.0%)	0 (0.0%)	
Depth of Invasion (DOI)					
Superficial/Moderate (≤10 mm)	30 (60.0%)	20 (60.6%)	5 (50.0%)	5 (71.4%)	0.669
Deep (>10 mm)	20 (40.0%)	13 (39.4%)	5 (50.0%)	2 (28.6%)	

[Table/Fig-7]: Association of PNI density (low density vs high density) with clinicopathological features.

p <0.001). Cases with intratumoural PNI also exhibited higher proportions of advanced T-stage (pT3/pT4a: 76.5% vs. 60.6%; p=0.351) and LVI (11.8% vs. 0.0%; p=0.111), though these did not reach statistical threshold.

The PNI-positive cases by nerve involvement density into low density (1-3 involved nerves/section; n=10, 58.8%) and high density (>3 involved nerves/section; n=7, 41.2%) is categorised in [Table/Fig-7]. Both low-density (80.0%) and high-density PNI (85.7%) demonstrated a highly significant association with cervical nodal metastasis (pN+) compared to PNI-absent cases (18.2%; p < 0.001). Additionally, low-density PNI was significantly associated with concomitant LVI (20.0% vs. 0.0%; p=0.016). Advanced T-stage (pT3/pT4a) remained elevated across both low-density (80.0%) and high-density (71.4%) cohorts.

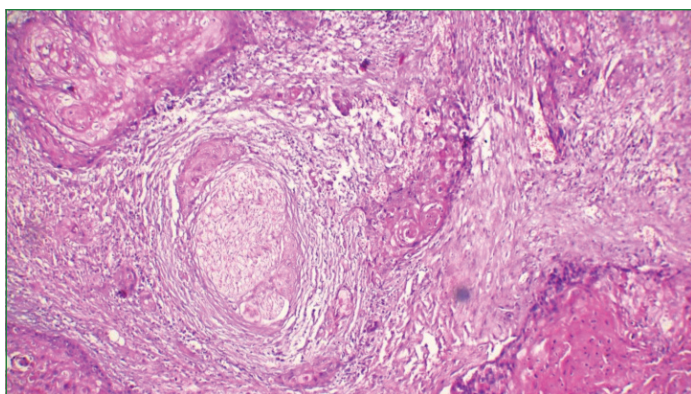
Microscopic evaluation of the positive cases confirmed classic features of PNI. High-power tissue examination clearly showed malignant epithelial cell sheets completely encircling a nerve bundle, tracking along the outer epineural sheath and involving more than 33% of its circumference [Table/Fig-8]. Under low-power tracking, these neurotropic events were found located completely within the central tumour bulk, confirming an intratumoural PNI structural distribution pattern according to the PNI grading framework [Table/Fig-9].



[Table/Fig-8]: High power showing Perineural Invasion (PNI) (H&E, x40).

DISCUSSION

The PNI represents an active neurotrophic process driven by complex paracrine interactions between malignant epithelial cells and peripheral nerve sheath elements within the tumour microenvironment. Although traditional histopathological examination relies on binary reporting (“present” vs. “absent”), binary assessment fails to capture microanatomical localisation and quantitative nerve density. To overcome these limitations, multidimensional framework such as the PNI classification system



[Table/Fig-9]: Low power showing Intratumoural Perineural Invasion (PNI) (H&E, x10).

incorporate spatial depth (intratumoural vs. extratumoural) and nerve involvement density (low-density vs. high-density) to refine risk stratification.

In the present study, a group of 50 primary OSCC cases, the overall prevalence of PNI was 34.0%. When measured strictly against other primary OSCC study groups, this prevalence aligns closely with established regional and international data. For example, Deepthi G et al., reported a PNI prevalence of 31.8% in a South Indian primary OSCC cohort, while Martínez-Flores R et al., observed PNI in 49.1% of primary OSCC resections [14,15]. Similarly, Varsha BK et al., identified PNI in 42.5% of primary OSCC cases [16]. Variations across primary OSCC studies are largely attributable to differences in slide sampling density, section thickness, and microscopic evaluation protocols.

Regarding microanatomical location, 100% of PNI-positive cases in the present study demonstrated intratumoural PNI, with no cases exhibiting extratumoural or peripheral nerve involvement. This spatial distribution mirrors patterns reported by Varsha BK et al., in primary OSCC, where the majority of PNI foci were confined within the central tumour mass [16]. In contrast, studies by Arun I et al., [17] and Martínez-Flores R et al., [15] observed extratumoural PNI in a subset of advanced-stage primary OSCC cases. The restriction of PNI to intratumoural zones in our cohort likely reflects the predominant inclusion of early-to-intermediate pathological T-stages (pT1–pT3 accounting for 74.0% of cases) and standard surgical margin clearance in primary wide local excisions.

Quantitatively, 58.8% of PNI-positive cases exhibited low-density PNI (1–3 involved nerves per section), while 41.2% demonstrated high-density PNI (>3 involved nerves per section). This distribution matches quantitative trends observed by Goswami PR and Singh G and Varsha BK et al., in primary OSCC, where low-density nerve involvement predominated in the majority of nerve-positive specimens [4,16].

The present study observed a statistically significant association between PNI presence and regional cervical lymph node metastasis (pN+) ($p < 0.001$). Nodal positivity was present in 82.4% of PNI-positive cases compared to 18.2% in PNI-absent cases. High nodal metastasis rates persisted across both low-density (80.0%) and high-density (85.7%) PNI subgroups ($p < 0.001$). Low-density PNI was also significantly associated with LVI ($p = 0.016$). These findings agree with primary OSCC studies by Laske RD et al., and Martínez-Flores R et al., confirming a strong link between nerve sheath infiltration and lymphatic dissemination [12,15]. Conversely, no statistically significant associations were identified between PNI and WHO histological grade ($p = 0.136$) or pathological T-stage ($p = 0.351$) within the present cohort.

Limitation(s)

The present study is limited by its cross-sectional design and small sample size, which restricted statistical analysis to univariate analysis without multivariable logistic regression or odds ratio estimation, preventing the confirmation of PNI as an independent prognostic factor and precluded long-term survival analyses or disease-free recurrence calculations. However, strict inclusion criteria and a standardised assessment system minimised observational bias. Further multicentre prospective trials with larger cohorts and longer follow-up durations are required to validate these findings and delineate the molecular mechanisms of neurotropic spread.

CONCLUSION(S)

In primary OSCC, PNI evaluated via the composite PNI classification frameworks serves as a strong indicator of aggressive tumour behaviour, demonstrating significant associations with regional cervical lymph node metastasis and LVI. Although univariate analysis validates PNI as an adverse pathological parameter, multivariable modeling in larger prospective cohorts is required to establish its independent prognostic value. Routine qualitative and quantitative PNI reporting on standardised H&E sections provides valuable risk stratification to assist multidisciplinary teams in postsurgical treatment planning.

REFERENCES

- [1] Muralidharan S, Nikalje M, Subramaniam T, Koshy JA, Koshy AV, Bangera D. A narrative review on oral squamous cell carcinoma. *J Pharm Bioallied Sci.* 2025;17(Suppl 1):S204-S206.
- [2] Binmadi N, Alsharif M, Almazrooa S, Aljohani S, Akeel S, Osailan S, et al. Perineural invasion is a significant prognostic factor in oral squamous cell carcinoma: A systematic review and meta-analysis. *Diagnostics.* 2023;13(21):3339.
- [3] Tao ZY, Chu G, Su YX. The prognostic role of perineural invasion for survival in head and neck squamous cell carcinoma: A systematic review and meta-analysis. *Cancers.* 2024;16(14):2514.
- [4] Goswami PR, Singh G. Perineural invasion (PNI): Definition, histopathological parameters of PNI in oral squamous cell carcinoma with molecular insight and prognostic significance. *Cureus.* 2023;15(6):e40123.
- [5] Quintana DMVO, Dedivitis RA, Kowalski LP. Prognostic impact of perineural invasion in oral cancer: A systematic review. *Acta Otorhinolaryngol Ital.* 2022;42(1):17–25. doi:10.14639/0392-100X-meta-4312.
- [6] Miller ME, Palla B, Chen Q, Elashoff DA, Abemayor E, St John MA, et al. A novel classification system for perineural invasion in noncutaneous head and neck squamous cell carcinoma: Histologic subcategories and patient outcomes. *Am J Otolaryngol.* 2012;33(2):212–215.
- [7] Singh P, Imam ZS, Singh S, Sinha S, Singh V, Sharma AK. Peri-neural invasion in oral squamous cell carcinoma: A clinico-pathological marker of OSCC prognosis and risk assessment. *J Adv Med Med Res.* 2021;33(17):1–9. Doi: 10.9734/jammr/2021/v33i1731020.
- [8] Sahoo A, Nazir A, Chandrashekar SP, Vyas T, Sikdar SD, Paul M, et al. Evaluating the diagnosis of cervical lymph node metastasis in oral squamous cell carcinoma by clinicopathologic investigation. *J Pharm Bioallied Sci.* 2025;17(Suppl 4):S2764-S2766.
- [9] WHO Classification of Tumours Editorial Board. Head and neck tumours. 5th ed. Lyon (France): International Agency for Research on Cancer; 2022.
- [10] Amin MB, Edge SB, Greene FL, Byrd DR, Brookland RK, Washington MK, et al., editors. *AJCC Cancer Staging Manual.* 8th ed. Chicago (IL): American Joint Committee on Cancer; Springer; 2017.
- [11] Liebig C, Ayala G, Wilks JA, Berger DH, Albo D. Perineural invasion in cancer: a review of the literature. *Cancer.* 2009;115(15):3379–3391.
- [12] Laske RD, Scholz I, Ikenberg KK, Meerwein C, Vital DG, Studer G, et al. Perineural invasion in squamous cell carcinoma of the oral cavity: Histology, tumor stage, and outcome. *Laryngoscope Invest Otolaryngol.* 2016;1(1):13–18.
- [13] Schmidt LB, Scanlon CS, D'Silva NJ. Perineural invasion in head and neck cancer. *J Dent Res.* 2018;97(7):742–750.
- [14] Deepthi G, Shyam ND, Kumar GK, Narayan V, Paremala K, Preethi P. Characterization of perineural invasion in different histological grades and variants of oral squamous cell carcinoma. *J Oral Maxillofac Pathol.* 2020;24:57–63.
- [15] Martínez-Flores R, Gómez-Soto B, Lozano-Burgos C, Niklander SE, Lopes MA, González-Arriagada WA. Perineural invasion predicts poor survival and cervical lymph node metastasis in oral squamous cell carcinoma. *Med Oral Patol Oral Cir Bucal.* 2023;28(5):e496.

[16]

Varsha BK, Radhika MB, Makarla S, Kuriakose MA, Satya Kiran GV, Padmalatha GV. Perineural invasion in oral squamous cell carcinoma: Case series and review of literature. J Oral Maxillofac Pathol. 2015;19(3):335-341.

[17]

Arun I, Roy P, Jain PV, Hameed S, Manikantan K, Arun P. Subcategorization of perineural invasion and its impact on survival in patients with oral squamous cell carcinoma. Head Neck Pathol. 2023;17:383-392.

PARTICULARS OF CONTRIBUTORS:

1. Postgraduate, Department of Pathology, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India.
2. Professor and Head, Department of Pathology, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India.
3. Professor, Department of Pathology, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India.
4. Professor, Department of Otorhinolaryngology (ENT), Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. TN Suresh,
Professor and Head, Department of Pathology, Sri Devaraj Urs Medical College,
Kolar-563103, Karnataka, India.
E-mail: Sureshtn.path@gmail.com

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