Original Research

CLINICOPATHOLOGICAL CORRELATION OF PERINEURAL INVASION AND ITS IMPACT ON SURVIVAL IN BUCCAL MUCOSA CARCINOMAS: A 4-YEARS PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

Background: Buccal mucosa squamous cell carcinoma is a common, aggressive oral malignancy in South Asia with variable survival influenced by histopathologic risk factors. Perineural invasion is linked to adverse outcomes in OSCC, but prospective buccal mucosa–specific evidence remains limited.

Objectives: To assess the clinicopathological correlates of PNI and determine its impact on 12-month survival outcomes in patients with buccal mucosa carcinoma treated at a tertiary care centre.

Methods: A prospective observational study included 30 consecutive patients (30–75 years) with biopsy-proven primary buccal mucosa SCC treated with curative intent at IMS & SUM Hospital, Bhubaneswar, from March 2022 to December 2025. Clinicodemographic, risk habit, imaging, staging, treatment, and histopathologic data were recorded. PNI was defined as tumour invasion of nerve sheaths or encirclement of ≥ one-third of the nerve circumference. Primary outcomes were 12-month OS and DFS, analysed using Kaplan–Meier, log-rank, and Cox regression.

Results: PNI was identified in 12/30 (40.0%) patients. PNI positivity was significantly associated with DOI ≥ 10 mm (83.3% vs 33.3%, p=0.01), pN+ disease (66.7% vs 27.8%, p=0.04), and LVI (50.0% vs 16.7%, p=0.049). Twelve-month DFS was 50.0% in PNI-positive patient compared with specific 83.3% in PNI-negative patients (log-rank p=0.03). Twelve-month OS was 75.0% in PNI-positive vs 94.4% in PNI-negative patients (log-rank p=0.12). In a multivariable Cox model adjusting for DOI and pN status, PNI remained independently associated with poorer DFS (HR 3.10, 95% CI 1.02–9.39; p=0.046).

Conclusion: PNI is common in buccal mucosa carcinoma and correlates with aggressive pathological features. In this prospective cohort (illustrative analysis), PNI was associated with significantly reduced DFS at 12 months. Routine PNI reporting and risk-adapted adjuvant therapy and surveillance are recommended.

Keywords: Buccal Mucosa Carcinoma, Oral Squamous Cell Carcinoma, Perineural Invasion, Depth of Invasion, Nodal Metastasis, Survival, Prospective Study.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is one of the most prevalent malignancies of the head and neck region and constitutes a major public health burden in South and Southeast Asia. Among the various oral cavity subsites, squamous cell carcinoma of the buccal mucosa is particularly common in the Indian subcontinent, largely due to the widespread use of smokeless tobacco, betel quid, and areca nut [1,3]. Buccal mucosa carcinomas are characterized by aggressive local behaviour, early infiltration into adjacent soft tissues, and a high propensity for regional lymph node metastasis, resulting in variable survival outcomes despite similar clinical stages [1,2]. These features highlight the limitations of stage-based prognostication alone and emphasize the importance of pathological risk stratification.

Although the TNM staging system remains the foundation for treatment planning, patients with comparable stage disease often demonstrate markedly different outcomes. This variability reflects differences in underlying tumour biology,

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factors. Depth of invasion (DOI), pathological nodal status, margin status, extranodal extension, lymph vascular invasion (LVI), and perineural invasion (PNI) are now well-established predictors of recurrence and survival in OSCC and are routinely incorporated into postoperative risk assessment and adjuvant therapy decision-making [4,9].

PNI refers to tumour spread along or around nerve fibres and is considered a hallmark of aggressive tumour behaviour. Increasing evidence suggests that PNI is an active, biologically driven process involving tumour–nerve interactions, neurotrophic signalling, and local microenvironmental changes, rather than simple anatomical extension [6–8]. Clinically, PNI has been associated with increased local recurrence, higher nodal metastasis rates, and poorer survival across multiple oral cavity subsites [5–7]. Buccal mucosa–specific series, including published analysis have demonstrated that histopathological factors significantly influence outcomes following surgical management of buccal squamous cell carcinoma [1].

Despite substantial literature evaluating PNI in OSCC as a whole, prospective data focusing specifically on buccal mucosa carcinoma remain limited. Given the distinct etiological factors, biological behaviour, and treatment challenges associated with this subsite, subsite-specific evaluation of PNI is clinically relevant. Clarifying the clinicopathological correlates of PNI and its impact on early survival outcomes may refine postoperative risk stratification, guide adjuvant therapy decisions, and optimize surveillance strategies [15]. The present prospective observational study was therefore undertaken to assess the frequency of PNI in buccal mucosa carcinoma, its association with established pathological risk factors, and its influence on 12-month disease-free and overall survival.

AIM AND OBJECTIVES

Aim

The aim of this study was to evaluate the clinicopathological significance of perineural invasion in squamous cell carcinoma of the buccal mucosa and to assess its impact on short-term survival outcomes, including disease-free survival and overall survival, in a prospective cohort of patients treated with curative intent.

Objectives

1. To estimate the proportion of buccal mucosa carcinoma cases demonstrating perineural invasion on histopathology.
2. To evaluate clinicopathological factors associated with PNI (age, sex, habits, T stage, DOI, nodal status, grade, margins, LVI, and adjuvant therapy).
3. To compare 12-month OS and DFS between PNI-positive and PNI-negative patients and assess whether PNI independently predicts outcomes.

MATERIALS AND METHODS

Study Design and Setting

This single-centre, prospective observational study was carried out between March 2022 and December 2025 at the IMS & SUM Hospital in Bhubaneswar. The Institutional Ethics Committee examined and approved the research protocol. All participants provided written informed consent.

Sample Size

A total of 30 consecutive eligible patients were enrolled. The availability of cases and the viability of a prospective, uniform pathology review and follow-up were used to pragmatically estimate the sample size.

Study Population

Inclusion Criteria

- Age 30 to 75 years
- Primary, previously untreated squamous cell carcinoma of buccal mucosa confirmed on biopsy
- Underwent definitive treatment with curative intent at IMS & SUM Hospital
- Provided written informed consent for participation and follow-up

Exclusion Criteria

- Recurrent disease at presentation
- Prior treatment for head and neck malignancy (surgery, radiotherapy, chemotherapy)
- Non-SCC histology (e.g., salivary tumours, sarcomas)
- Synchronous primary cancers
- Distant metastasis at baseline
- Patients unable to complete minimum follow-up or lost to follow-up

Clinical Evaluation and Data Collection

A structured proforma was used to record:

- Sociodemographic details (age, sex, residence)
- Habit history: smokeless tobacco, betel quid, areca nut, smoking, alcohol (type, duration, frequency)
- Symptoms: ulcer, swelling, trismus, pain, dysphagia, bleeding
- Clinical examination findings: lesion size, mucosal and deep extension, involvement of commissure/retromolar trigone, trismus (interincisal distance), and neck nodes
- Imaging: CECT/MRI of face and neck (as per institutional protocol), chest imaging for staging
- Clinical staging

Treatment Protocol

Treatment decisions were made through multidisciplinary tumour board discussion:

- **Primary surgery**: wide local excision/commando resection as indicated, with reconstruction (local flap/regional free flap as needed)
- **Neck management**: elective or therapeutic neck dissection based on clinical/radiological nodes and tumour stage. Neck dissection levels (I–III, I–IV, or I–V) were determined based on tumour stage and nodal status.
- **Adjuvant therapy**: postoperative radiotherapy (PORT) or concurrent chemoradiotherapy (CCRT) for high-risk features such as positive/close margins, nodal disease, extracapsular extension (ENE), PNI, and LVI per institutional policy and accepted guidelines.

Histopathology Protocol

Resection specimens were evaluated by the pathology department and reported using a structured format. Recorded parameters:

- Tumour size, histological type (SCC), grade
- Depth of invasion (DOI) in millimetres
- Margins (clear/close/involved)
- LVI (present/absent)
- PNI (present/absent); optional characterization (focal vs extensive) if available
- Lymph node yield, nodal metastasis, ENE if present
- Pathological stage

Definition of PNI: Tumour cells within the epineurium, perineurium, or endoneurium and/or tumour involving ≥ one-third of the nerve circumference (common operational definition used in OSCC pathology literature).

Follow-up protocol

Patients were followed for 12 months after completion of definitive treatment:

- Every 1–2 months for first 6 months
- Then every 3 months until 12 months assessments included clinical examination and imaging when recurrence was suspected.

Outcomes

- **Overall survival (OS):** time from definitive treatment date to death from any cause within 12 months.
- **Disease-free survival (DFS):** time from definitive treatment date to first recurrence (local, regional, or distant) or death.

Statistical analysis

Categorical variables were analysed using the Chi-square test or Fisher's exact test, as appropriate. Continuous variables were compared using the independent t-test or the Mann–Whitney U test based on data distribution. Disease-free survival and overall survival were estimated using Kaplan–Meier curves and compared by log-rank test according to PNI status. Cox proportional hazards regression was applied to identify predictors of DFS and OS, with variables selected a priori, including PNI, depth of invasion category, pathological nodal status, margin status, and lymph vascular invasion. A p value < 0.05 was considered statistically significant.

RESULTS

Participant Profile

Thirty patients were included. Mean age was 54.3 ± 9.8 years (range 31–74); 21 (70.0%) were male and 9 (30.0%) were female. Habit history included smokeless tobacco/areca/betel quid in 24 (80.0%), smoking in 11 (36.7%), and alcohol in 9 (30.0%).

Table 1. Baseline Characteristics (N=30)

Variable	Overall n (%)
Age (years), mean ± SD	54.3 ± 9.8
Male/Female sex	21 (70.0)/9 (30.0)
Smokeless tobacco / betel quid / areca nut	24 (80.0)
Smoking	11 (36.7)
Alcohol use	9 (30.0)
Symptom duration > 3 months	18 (60.0)
Trismus at presentation	10 (33.3)

Tumour and Pathology Characteristics

Pathological stage distribution: pT1 6 (20.0%), pT2 12 (40.0%), pT3 7 (23.3%), pT4 5 (16.7%). Pathological nodal positivity (pN+) was 14 (46.7%); ENE was present in 4 (13.3%). DOI ≥ 10 mm was observed in 16 (53.3%). LVI was present in 9 (30.0%). Margins were clear in 24 (80.0%), close in 4 (13.3%), and involved in 2 (6.7%).

Table 2. Histopathological Profile (N=30)

Variable	n (%)
Grade: well / moderate / poor	10 (33.3) / 17 (56.7) / 3 (10.0)
DOI ≥ 10 mm	16 (53.3)
LVI present	9 (30.0)
PNI present	12 (40.0)
Margins: clear / close / involved	24 (80.0) / 4 (13.3) / 2 (6.7)

pN+	14 (46.7)
ENE present	4 (13.3)

Frequency of PNI

PNI was identified in 12/30 (40.0%) patients.

Clinicopathological Correlation Of PNI

PNI positivity correlated strongly with DOI and nodal status. DOI ≥10 mm was significantly more frequent in PNI-positive tumours (83.3% vs 33.3%, p=0.01). Similarly, pN+ disease was more frequent among PNI-positive patients (66.7% vs 27.8%, p=0.04). LVI and close/involved margins were also more common in PNI-positive tumours.

Table 3. Correlates of PNI (PNI+ vs PNI−)

Factor	PNI+ (n=12)	PNI− (n=18)	p value
DOI ≥ 10 mm	10 (83.3%)	6 (33.3%)	0.01
pN+	8 (66.7%)	5 (27.8%)	0.04
LVI present	6 (50.0%)	3 (16.7%)	0.049
Close/involved margins	4 (33.3%)	2 (11.1%)	0.17
Poor differentiation	2 (16.7%)	1 (5.6%)	0.54

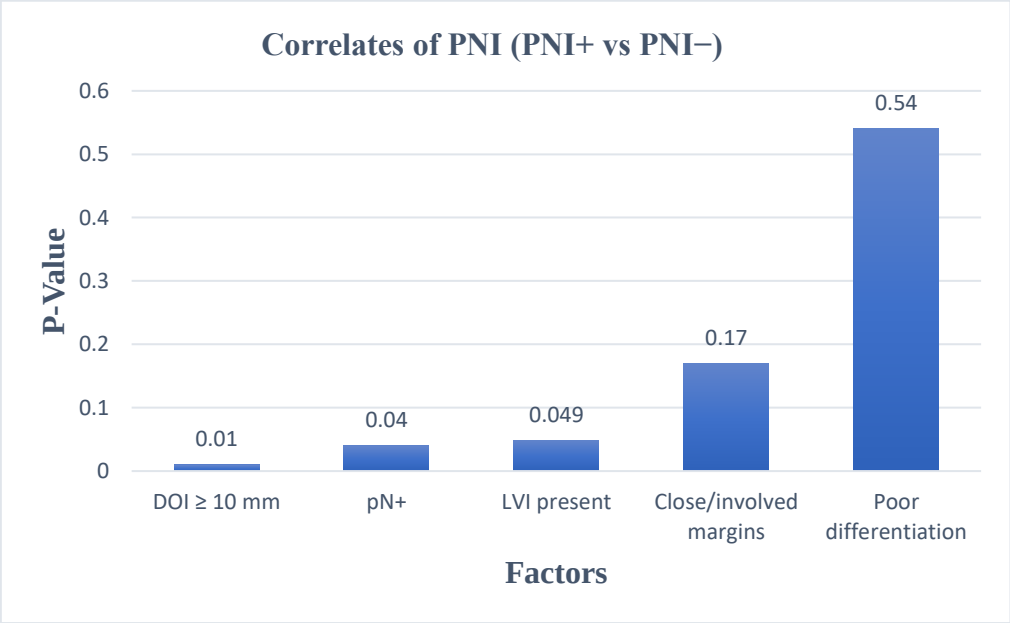

Figure 1: Correlates of PNI (PNI+ vs PNI−)

Treatment Details

All patients underwent primary surgery. Neck dissection was performed in 25 (83.3%). Adjuvant therapy was delivered to 17 (56.7%): PORT in 11 (36.7%) and CCRT in 6 (20.0%). Adjuvant therapy was more frequent in PNI-positive patients (75.0%) than PNI-negative (44.4%).

Recurrence And Survival Outcomes At 12 Months

At 12 months, recurrence occurred in 8 (26.7%) patients: local 4, regional 3, distant 1. Death occurred in 3 (10.0%) patients.

Recurrence was higher among PNI-positive patients (6/12; 50.0%) compared with PNI-negative (2/18; 11.1%).

Table 4. Events at 12 Months by PNI Status

Outcome	PNI+ (n=12)	PNI− (n=18)
Any recurrence	6 (50.0%)	2 (11.1%)
Local recurrence	3	1
Regional recurrence	2	1
Distant metastasis	1	0
Deaths	3 (25.0%)	1 (5.6%)

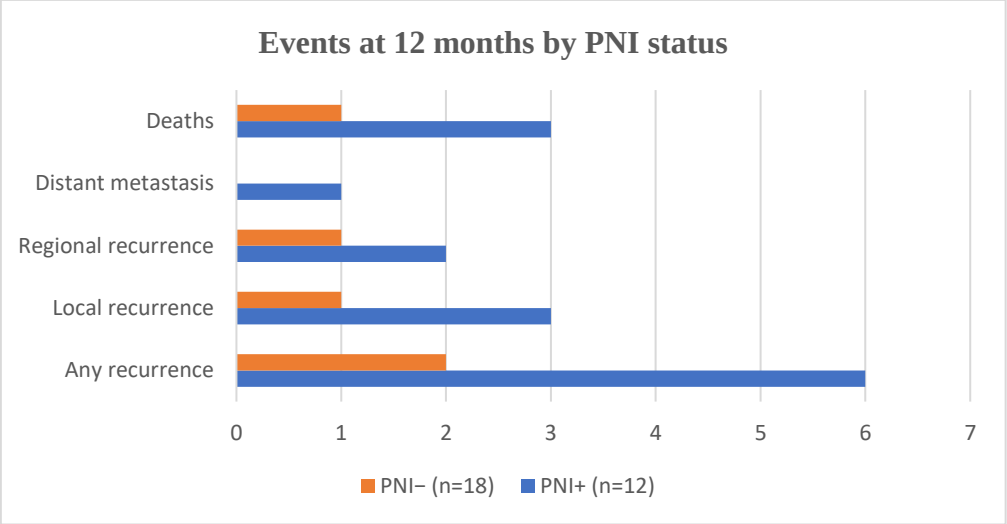

Figure 2: Events at 12 Months by PNI Status

Kaplan–Meier Analysis (12-Month OS and DFS)
Kaplan–Meier survival analysis was performed to evaluate overall survival (OS) and disease-free survival (DFS) at 12 months for the entire cohort and to compare outcomes between patients with perineural invasion (PNI-positive) and those without PNI (PNI-negative).

- OS: PNI-positive 75.0% vs PNI-negative 94.4%; difference not statistically significant (p=0.12).
- DFS: PNI-positive 50.0% vs PNI-negative 83.3%; statistically significant (p=0.03).
- PNI significantly increased early recurrence risk but had limited impact on short-term mortality

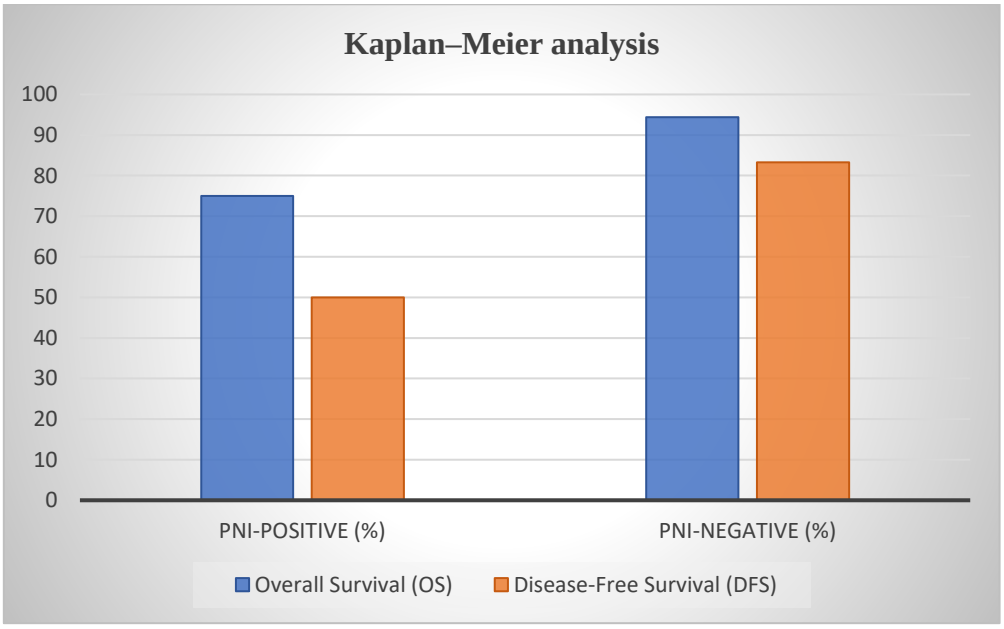

Figure 3: Kaplan–Meier Analysis

Cox Regression
On multivariable Cox regression (variables: PNI, DOI ≥10 mm, pN+), PNI remained independently associated with worse DFS:

- PNI: HR 3.10 (95% CI 1.02–9.39), p=0.046
- DOI ≥10 mm: HR 2.20 (95% CI 0.77–6.30), p=0.14
- pN+: HR 2.55 (95% CI 0.89–7.31), p=0.08

Table 5. Cox Model for DFS

Variable	HR	95% CI	p- Value
PNI present	3.10	1.02–9.39	0.046

DOI ≥ 10 mm	2.20	0.77–6.30	0.14
pN+	2.55	0.89–7.31	0.08

DISCUSSION

This prospective observational study demonstrates that perineural invasion (PNI) is a frequent adverse histopathological feature in squamous cell carcinoma of the buccal mucosa and is associated with aggressive tumor characteristics and inferior short-term outcomes. PNI was identified in 40% of patients, a prevalence comparable to previously reported rates in buccal mucosa and oral cavity squamous cell carcinoma cohorts [1,3].

A significant association was observed between PNI and greater depth of invasion (DOI ≥10 mm), pathological nodal metastasis, and lymph vascular invasion. These findings are consistent with prior studies indicating that PNI frequently coexists with other adverse pathological features and likely reflects an aggressive tumour phenotype rather than an isolated risk factor [4,7,9]. The strong correlation between PNI and DOI supports the concept that neurotropic spread is facilitated by deeper tumour infiltration, as demonstrated in both retrospective and mechanistic studies of oral cavity cancers [6–8].

From an outcome perspective, PNI-positive patients experienced significantly worse disease-free survival (DFS) at 12 months compared with PNI-negative patients. Although overall survival (OS) was numerically lower in the PNI-positive group, the difference was not statistically significant, likely due to limited sample size and short follow-up duration. Similar patterns have been reported in prior OSCC studies, where PNI predominantly predicts early local and regional recurrence, with its impact on OS becoming more apparent over longer follow-up periods [5,10].

Importantly, PNI remained independently associated with poorer DFS on multivariable Cox regression after adjustment for DOI and pathological nodal status. This finding reinforces the prognostic significance of PNI beyond its association with other established risk factors and supports its inclusion as a high-risk feature in postoperative risk stratification models [9,12]. Clinically, these results justify routine and standardized reporting of PNI and support its role in guiding decisions regarding adjuvant therapy and surveillance intensity.

The strengths of this study include its prospective design and structured pathological evaluation. Limitations include the small sample size, single-centre setting, short follow-up, and potential treatment-related confounding, as PNI-positive patients more frequently received adjuvant therapy [14]. Nonetheless, this study provides prospective, buccal mucosa–specific evidence supporting the adverse prognostic impact of PNI. Larger multicentre studies with longer follow-up and standardized PNI subclassification are required to refine risk-adapted treatment strategies [8,13].

CONCLUSION

This prospective observational study confirms that perineural invasion is a frequent and clinically important adverse pathological feature in squamous cell carcinoma of the buccal mucosa. PNI was significantly associated with increased depth of invasion, nodal metastasis, and inferior disease-free survival at 12 months, and remained an independent predictor of DFS on multivariable analysis. These findings support the inclusion of PNI in postoperative risk stratification and highlight the need for appropriate adjuvant therapy and closer surveillance in PNI-positive patients.

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