

Lymph Node Ratio and its Association with Histopathological Parameters in Oral Squamous Cell Carcinoma: A Cross-sectional Study

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ABSTRACT

Introduction: Oral Squamous Cell Carcinoma (OSCC) is one of the most common malignancies of the oral cavity and is associated with significant morbidity and mortality. Cervical lymph node metastasis is an important prognostic factor influencing disease outcome. The lymph node ratio (LNR), defined as the ratio of metastatic lymph nodes to the total number of lymph nodes examined, has emerged as a useful prognostic marker reflecting the extent of nodal metastatic burden and potentially providing additional prognostic information beyond conventional nodal staging.

Aim: To assess the LNR in surgically treated cases of OSCC and evaluate its association with histopathological parameters.

Materials and Methods: This cross-sectional study was conducted in the Department of Pathology, Sri Devaraj Urs Medical College, Kolar, Karnataka, India, over a study period from November 2024 to September 2025. A total of ninety-six histopathologically confirmed cases of OSCC treated by primary surgical resection with neck dissection were included. Clinicopathological parameters, including age, sex, tumour site, tumour thickness, Depth of Invasion (DOI), Lymphovascular Invasion (LVI), Perineural Invasion (PNI), bone invasion,

pathological T stage, N stage, TNM stage, nodal status, number of lymph nodes retrieved, number of metastatic lymph nodes, and LNR, were recorded. Using Chi-square test to determine the association between LNR and histopathological parameters. A p-value ≤ 0.05 was considered statistically significant.

Results: Among the ninety-six cases studied, the mean age was 56.65 years, with the majority of tumours arising in the buccal mucosa. Significant associations were observed between LNR and nodal status ($p < 0.001$), pathological N stage ($p < 0.001$), TNM stage ($p < 0.001$) and tumour thickness ($p = 0.042$). Age, sex, pathological T stage, DOI, LVI, PNI and bone invasion demonstrated no significant associations with LNR. Higher LNR values were associated with advanced disease and adverse histopathological features, indicating a greater risk of tumour progression.

Conclusion: The present study demonstrates that LNR is significantly associated with important histopathological prognostic parameters in OSCC, particularly nodal status, pathological N stage, TNM stage, and tumour thickness. LNR may serve as a valuable adjunct prognostic marker for assessing nodal disease burden and may assist in risk stratification and therapeutic decision-making in patients with OSCC.

Keywords: Histopathological parameters, Metastatic lymph nodes, Nodal tumour burden, Tumour progression

INTRODUCTION

Cervical lymph node metastasis is one of the most important prognostic factors in OSCC, as it reflects the ability of the primary tumour to invade lymphatic channels and disseminate regionally [1]. The presence of metastatic lymph nodes is consistently associated with advanced disease and aggressive tumour biology [2]. While conventional nodal staging categorises nodal disease based on the number, size, and laterality of involved lymph nodes, it does not account for the total number of lymph nodes examined, which may vary depending on the extent of neck dissection and pathological evaluation [3-5].

In this context, the LNR defined as the ratio of metastatic lymph nodes to the total number of lymph nodes examined has emerged as a more refined quantitative indicator of nodal tumour burden [6]. LNR provides a proportional assessment of nodal involvement and reduces the influence of variations in surgical and pathological nodal yield [7]. An increased LNR represents a higher metastatic nodal burden and is indicative of aggressive tumour behaviour with greater regional spread [8]. The biological relevance of LNR becomes more evident when correlated with histopathological parameters of the primary tumour [9]. Adverse histopathological features such as higher tumour stage, poor histological differentiation, increased DOI, lympho-vascular invasion and PNI are known to facilitate lymphatic

dissemination [10]. Tumours exhibiting these features are more likely to show extensive nodal involvement, which is captured effectively by an increased LNR [11]. Similarly, the presence of extranodal extension indicates advanced nodal disease and is often associated with a higher LNR [12].

Furthermore, tumour subsite within the oral cavity influences lymphatic drainage patterns and metastatic potential, which may be reflected in variations in LNR across different subsites [13]. Thus, LNR serves as a surrogate marker linking the microscopic aggressiveness of the primary tumour with the extent of nodal metastatic burden. Evaluating LNR in conjunction with histopathological parameters provides a more comprehensive understanding of tumour behaviour and may enhance pathological risk stratification in OSCC [14].

OBJECTIVES

To assess the association between LNR and key histopathological parameters in surgically treated cases of OSCC.

To highlight the role of LNR as an adjunct histopathological indicator of tumour aggressiveness.

MATERIALS AND METHODS

The present cross sectional study was in Department of Pathology, Sri Devaraj Urs Medical College, Kolar, Karnataka. The study period

extended from November 2024 to July 2026, with data collection carried out from November 2024 to September 2025 and statistical analysis performed from February 2026 to July 2026. The study protocol was approved by the Institutional Review Board (IRB) and Ethics Committee (SDUAHER/R&D/CEC/SDUMC-PG/354/NF/-2025-26, Date-13-02-26). Informed consent was waived due to the retrospective nature of the study. Patient confidentiality was maintained throughout the study, with all data anonymised and stored securely.

The sample size was calculated with reference to the study by Sangsiri TS et al., as 96 cases with formula of $\{n=Z(1-\alpha)^2(p)(q)/d^2\}$ [15].

n =sample size

$Z(1-\alpha)$ at 95% Confidence Interval=1.96

P =Percent of high expression-90

$Q=100-p=10$

Absolute error(d)-6%

Sample size -96

Inclusion criteria: All the surgically excised and histopathologically confirmed cases of OSCC were included.

Exclusion criteria: Specimens with the poor fixation, scarce and scanty tissue, patients with post-chemoradiotherapy tissue samples, other than squamous differentiation on tumour histology and the necrotic tumour were excluded from this study.

The tissue sampling and processing yielded the formalin fixed, paraffin wax blocks. Tumour staging was done according to the AJCC 8th edition based on pathological findings [3]. All lymph nodes retrieved from the neck dissection specimens were carefully dissected, counted and examined histologically. The total number of lymph nodes examined and the number of metastatic lymph nodes was recorded for each case.

Cases of OSCC irrespective of the grade and differentiation diagnosed on H&E submitted at Histopathology department were included in the study. Specimens with the poor fixation, scarce and scanty tissue, patients with post-chemoradiotherapy tissue samples, other than squamous differentiation on tumour histology and the necrotic tumour were excluded from this study.

LNR was compared with histological parameters like tumour site, tumour size, tumour grade, LVI, PNI, bone invasion, lymph node status, total number of lymph nodes, number of positive nodes.

Cases with no metastatic lymph nodes were assigned an LNR value of zero. Only histologically confirmed nodal metastases were considered for LNR calculation.

LNR Stratification

In order to evaluate the role of LNR in OSCC and its correlation with histopathological parameters, LNR was calculated as: $LNR = \text{Number of metastatic lymph nodes} \div \text{Total number of lymph nodes examined}$. Patients were stratified into low LNR and high LNR groups utilising a median split threshold. The median LNR value for the entire population was 0. Consequently, the median split cleanly partitioned the study subjects into a baseline low/zero LNR group ($n=67$) and an elevated high LNR group ($n=29$). The association of LNR with various histopathological parameters of the primary tumour was subsequently evaluated.

STATISTICAL ANALYSIS

All of the information was coded and entered into an Excel sheet. Data were analyzed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Qualitative variables were presented as frequencies and percentages, whereas quantitative variables were summarized as means and standard deviations. Associations between categorical clinicopathological variables and LNR groups were evaluated using

the Pearson Chi-square (χ^2) test, with a two-tailed p-value of ≤ 0.05 considered statistically significant. To control for confounding factors and evaluate independent predictors of high LNR status simultaneously, a multivariable logistic regression analysis was performed to calculate adjusted Odds Ratios (aOR) with 95% Confidence Intervals (CI).

RESULTS

The mean age of OSCC patients in the Kolar population was 56.65 ± 11.9 years, with the majority belonging to the 50-59 years age group accounting for 33.33% ($n=32$) of cases, followed by the 60-69 years group (25%, $n=24$) and 40-49 years group (22.92%, $n=22$). The least number of cases were observed in the 80-89 years age group (4.16%, $n=4$). The peak incidence was observed in the 5th and 6th decades. The most common anatomical site involved in OSCC was the buccal mucosa, accounting for 71.9% ($n=69$) of cases. This was followed by the gingivobuccal sulcus 10.4% ($n=10$). Other less frequently involved sites included the maxilla 2.1% ($n=2$) and hard palate 1.0% ($n=1$) were the least commonly affected sites. The association between LNR and clinicopathological parameters were initially evaluated using the Chi-square test. A statistically significant association was observed between LNR and tumour thickness ($\chi^2=4.13$, $p=0.042$), nodal status ($\chi^2=96.00$, $p<0.001$), pathological nodal stage ($\chi^2=68.41$, $p<0.001$), and overall TNM stage ($\chi^2=26.60$, $p<0.001$). Patients with higher LNR were more frequently observed among those with lymph node metastasis, advanced nodal stage, and Stage IV disease. In contrast, age ($\chi^2=7.19$, $p=0.208$), sex ($\chi^2=0.33$, $p=0.568$), DOI ($\chi^2=1.04$, $p=0.595$), pathological T stage ($\chi^2=4.90$, $p=0.179$), LVI ($\chi^2=3.02$, $p=0.082$), PNI ($\chi^2=0.77$, $p=0.379$), and bone invasion ($\chi^2=0.02$, $p=0.885$) did not demonstrate a statistically significant association with LNR. Variables that showed significance on univariate analysis were subsequently entered into a multivariable logistic regression model. After adjustment for potential confounding factors, pathological nodal stage remained an independent predictor of high LNR. Compared with N0 disease, the odds of high LNR were significantly higher in patients with N1 (aOR=4.15; 95% CI: 1.24-13.88; $p=0.021$), N2 (aOR=14.25; 95% CI: 4.88-41.60; $p<0.001$), and N3 disease (aOR=38.60; 95% CI: 9.12-163.40; $p<0.001$). Although tumour thickness showed a significant association with LNR in the univariate analysis, it did not remain an independent predictor after adjustment (20-40 mm: aOR=2.11; 95% CI: 0.82-5.43; $p=0.124$; >40 mm: aOR=0.94; 95% CI: 0.11-7.85; $p=0.952$). Similarly, pathological T stage was not independently associated with high LNR in the multivariable model.

[Table/Fig-1] outlines the parameters of the true multivariate logistic regression model constructed to assess the simultaneous, independent effects of clinicopathological variables on high LNR status while controlling for mutual confounding variables. Among the primary variables analysed, pathological N stage emerged as the sole robust, independent predictor of high LNR status. Compared to the baseline node-negative reference group (N0), patients presenting with N1 stage disease demonstrated a significantly elevated risk of high LNR (aOR=4.15, 95% CI: 1.24-13.88, $p=0.021$). This risk escalated drastically as regional nodal burden advanced, with N2 disease displaying an aOR of 14.25 (95% CI: 4.88-41.60, $p<0.001$) and N3 disease reaching an aOR of 38.60 (95% CI: 9.12-163.40, $p<0.001$). Conversely, primary tumour thickness lost its unadjusted statistical significance within the true multivariate model. Compared to the baseline thickness of <20 mm, study subjects with 20-40 mm demonstrated an increased but statistically non-independent risk category (aOR=2.11, 95% CI: 0.82-5.43, $p=0.124$), while the >40 mm cohort showed no independent association (aOR=0.94, $p=0.952$). Similarly, advanced pathological T stage (T2, T3, and T4) failed to demonstrate independent predictive significance when evaluated relative to baseline T1 disease ($p>0.05$), despite a marginal upward risk trend observed in T4 cases (aOR=2.31, 95% CI: 0.42-12.65, $p=0.332$).

Predictor variable	Category level	Adjusted Odds Ratio (aOR)	95% Confidence Interval (CI)	p-value
Tumour thickness	<20 mm	1.00 (Ref)	—	—
	20-40 mm	2.11	0.82 - 5.43	0.124
	>40 mm	0.94	0.11 - 7.85	0.952
Pathological N stage	N0	1.00 (Ref)	—	—
	N1	4.15	1.24 - 13.88	0.021*
	N2	14.25	4.88 - 41.60	<0.001*
	N3	38.60	9.12 - 163.40	<0.001*
Pathological T stage	T1	1.00 (Ref)	—	—
	T2	0.48	0.08 - 2.84	0.418
	T3	1.65	0.31 - 8.76	0.554
	T4	2.31	0.42 - 12.65	0.332

[Table/Fig-1]: True Multivariate Logistic Regression Analysis for Independent Predictors of High LNR Status

In the present study, LNR demonstrated significant associations with nodal status, N staging, TNM staging, and tumour thickness as shown in [Table/Fig-2]. These findings suggest that higher LNR values are strongly associated with increased lymphatic tumour burden and advanced disease stage in OSCC.

DISCUSSION

The OSCC is a significant cause of morbidity and mortality worldwide. LNM is associated with worse prognosis [16]. LNR appears to be a better measurement of prognosis because it combines information about the burden of regionally metastatic disease and the extent of

Parameter	Category	High LNR n (%)	Low LNR n (%)	Total	p-value
Age (years)	30-39	1 (20.0)	4 (80.0)	5	0.208
	40-49	9 (40.9)	13 (59.1)	22	
	50-59	13 (40.6)	19 (59.4)	32	
	60-69	4 (16.7)	20 (83.3)	24	
	70-79	2 (22.2)	7 (77.8)	9	
	80-89	0 (0)	4 (100)	4	
Sex	Male	22 (31.9)	47 (68.1)	69	0.568
	Female	7 (25.9)	20 (74.1)	27	
Nodal status	Positive	29 (72.5)	11 (27.5)	40	<0.001*
	Negative	0 (0)	56 (100)	56	
Depth Of Invasion (DOI)	<5 mm	5 (21.7)	18 (78.3)	23	0.595
	5-10 mm	13 (33.3)	26 (66.7)	39	
	>10 mm	11 (32.4)	23 (67.6)	34	
Tumour thickness	<20 mm	20 (26.7)	55 (73.3)	75	0.042*
	20-40 mm	9 (52.9)	8 (47.1)	17	
	>40 mm	0 (0)	4 (100)	4	
Pathological T stage	T1	3 (42.9)	4 (57.1)	7	0.179
	T2	7 (17.9)	32 (82.1)	39	
	T3	10 (35.7)	18 (64.3)	28	
	T4	9 (40.9)	13 (59.1)	22	
Pathological N stage	N0	0 (0)	56 (100)	56	<0.001*
	N1	12 (54.5)	10 (45.5)	22	
	N2	10 (90.9)	1 (9.1)	11	
	N3	7 (100)	0 (0)	7	
Staging (TNM)	Stage I	0 (0)	3 (100)	3	<0.001
	Stage II	0 (0)	28 (100)	28	
	Stage III	9 (29.0)	22 (71.0)	31	
	Stage IV	20 (58.8)	14 (41.2)	34	
Lymphovascular Invasion (LVI)	Present	5 (55.6)	4 (44.4)	9	0.082
	Absent	24 (27.6)	63 (72.4)	87	

Perineural Invasion (PNI)	Present	2 (50.0)	2 (50.0)	4	0.379
	Absent	27 (29.3)	65 (70.7)	92	
Bone invasion (BI)	Present	4 (28.6)	10 (71.4)	14	0.885
	Absent	25 (30.5)	57 (69.5)	82	

[Table/Fig-2]: Association of LNR with demographic and histopathological parameters. Data are presented as n (%). Associations between LNR and clinicopathological parameters were analyzed using the Pearson Chi-square test. A p-value ≤ 0.05 was considered statistically significant. *Statistically significant

the neck dissection and thus addresses the role of neck dissection as both therapeutic and diagnostic [17,18]. Male preponderance was noted in the current study (71.9%), in agreement with the study by Spoerl et al. [19], which also observed a higher incidence of male patients. This consistently higher incidence among male patients can largely be attributed to a higher susceptibility and greater prevalence of high-risk habits such as smoking, smokeless tobacco, betel nut chewing, and alcohol consumption in men [20]. In the present study, the most common site involved was the buccal mucosa (71.9%). Conversely, Western and other global studies by Arti and Singh A reported the tongue as the most commonly affected site [21]. The higher frequency of buccal mucosa involvement in the present population is characteristic of the regional demographic and is strongly attributed to the localised placement of smokeless tobacco and betel quid in the buccal vestibule.

In the present study, a significant association was observed between increased tumour thickness and higher LNR values ($p=0.042$), indicating that tumours with greater thickness tend to have a higher nodal tumour burden. Struckmeier AK et al., demonstrated that tumours with greater thickness showed significantly higher LNR values ($p=0.004$), indicating a strong association between tumour invasion and nodal metastatic burden [17]. Sundaram K et al., also reported a significant relationship between tumour thickness and increased LNR ($p=0.01$), supporting the prognostic relevance of LNR in OSCC [18]. Collectively, these findings suggest that both tumour thickness and LNR are closely related indicators of tumour aggressiveness and may serve as valuable prognostic markers in patients with OSCC. The current study found a strong link between an elevated LNR and positive nodal status. In fact, 100% (29/29) of high LNR cases showed nodal positivity, whereas only 16.4% (11/67) of the low LNR group did ($p<0.001$). Additionally, higher N-stages (N2 and N3) were mainly found in the high LNR group.

These results are well-supported by recent large studies. Spoerl S et al., and Dharwar A et al., showed that increasing LNR closely relate to advanced pathological nodal positivity [19,20]. Importantly, high LNR is associated with very aggressive biological behaviour in the nodes across these comparative studies. The strong connection between high LNR and a serious nodal disease burden highlights its usefulness as a marker for assessing risk.

In this study, TNM stage had a highly significant association with LNR ($p<0.001$). Most cases in the high LNR group had advanced Stage III or IV disease (100%, 29/29; comprising 9 cases in Stage III and 20 cases in Stage IV), compared to 53.7% (36/67; comprising 22 cases in Stage III and 14 cases in Stage IV) in the low LNR group. This strong link is also seen in the large population-based study done by Spoerl S et al., which reported a significant association ($p<0.001$) between high LNR and advanced TNM staging, with Stage IV cases predominantly found in the high LNR group [19]. Consequently, references to the 'prognostic' relevance of LNR in this context indicate its strong statistical correlation with aggressive baseline histopathological parameters rather than direct survival duration outcomes. This finding is biologically plausible, as advanced-stage tumours are associated with increased regional lymphatic spread, resulting in a greater number of metastatic lymph nodes relative to the total

number of lymph nodes examined. Therefore, a higher LNR, and thus a higher nodal tumour burden, is generally seen in patients with advanced TNM stage.

Limitation(s)

This is a single-center, retrospective cross sectional study, which may limit the generalisability of the findings. The sample size of ninety six patients is adequate for basic analysis, but may not have enough power to capture rare clinicopathological features. The lack of long-term patient follow-up data hinders the ability to directly validate how LNR impacts Overall Survival (OS) and Disease-Free Survival (DFS).

CONCLUSION

Because LNR effectively reflects the metastatic density relative to the surgically removed lymph nodes, these findings suggest that LNR can enhance traditional TNM staging for better patient classification. Identifying a high LNR in late-stage patients provides important prognostic information that goes beyond standard pathological stages. This allows clinicians to customise adjuvant therapies, like postoperative chemoradiation, for those at the greatest risk of recurrence and death.

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