Indian Journal of Otolaryngology and Head & Neck Surgery (2024) 76:3261–3270
https://doi.org/10.1007/s12070-024-04661-4

ORIGINAL ARTICLE

Prognostic Role of Tumor Budding and Worst Pattern of Invasion in Lymph Node Metastasis and Disease-Free Survival in Oral Squamous Cell Cancer Patients: Result from Central India's Regional Cancer Centre

Renuka Badanale[1] · Vandana Mohta[2] · Aniruddha Wagh[2] · Kartar Singh[3]

Received: 20 February 2024 / Accepted: 20 March 2024 / Published online: 12 April 2024

Abstract

Aim To study the role of pattern of invasion, tumor budding and other clinicopathological parameters in determining the risk of nodal metastases and disease-free survival in oral squamous cell cancer patients.

Method The data of 90 patients with oral squamous cell carcinoma who underwent surgery as their primary modality of treatment were retrospectively analysed. Predictive significance of clinicopathological parameters was assessed with Univariate analysis with Fisher exact test and unpaired t-test. The factors which were significant on Univariate analysis were then analysed with multivariate analysis using logistic regression model to find independent predictors. *P* value < 0.05 was considered significant. Disease free survival analysis was performed using Kaplan-Meier method and comparison done using the log-rank test for each group.

Result The age of the patients ranged from 22yrs to 72 years with male predominance (81.1%). The most common site of involvement was buccal mucosa. Significant factors predicting nodal metastases on univariate analysis were site ($p = 0.031$), grade ($p = 0.012$), T stage (p = < 0.001), Depth of invasion (p = < 0.001), perineural invasion (p = < 0.001), lymphovascular emboli ($p = 0.018$), tumor budding (p = < 0.001), pattern of invasion (p = < 0.001) and stroma ($p = 0.037$). On multivariate analysis tumor budding ($p = 0.016$), depth of invasion ($p = 0.016$) and perineural invasion ($p = 0.044$) were predictive of nodal metastasis. A statistically significant difference in 3year disease free survival was seen in infiltrative pattern of invasion and tumor budding which showed a *p*-value of 0.0372 and 0.0489 respectively.

Conclusion Based on the findings of the present study and review of previous articles tumor budding, worst pattern of invasion, host lymphocyte response should also be included in routine histopathology reporting of OSCC.

Key Message

Based on the findings of the present study tumor budding, worst pattern of invasion, host lymphocyte response should also be included in routine histopathology reporting of OSCC.

Keywords oral cancer · Tumor Budding · Worst Pattern of Invasion

Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common malignancies worldwide with significant mortality and morbidity. In our institution OSCC accounts for around 35% of total malignancies. Prognosis and treatment of OSCC mainly depends on tumor stage and nodal metastasis. Even early-stage tumors can show metastatic nodes and aggressive behaviour with fatal outcomes [1]. Despite the

✉ Vandana Mohta
vandanamohta@gmail.com

[1] Department of Pathology, Government Medical college, Nagpur, India

[2] Department of Head and neck Oncology, Rashtrasant Tukdoji Cancer Hospital, Nagpur, India

[3] Department of Radiation Oncology, Rashtrasant Tukdoji Cancer Hospital, Nagpur, India

advancements in diagnostic and management modalities, the mortality rates have remained static.

Lymph node (LN) status is one of the most important adverse prognostic factors leading to 50% decrease in survival with higher incidence of distant metastasis.[2] Clinical examination and imaging modalities are being used to detect preoperative nodal status; however with only 70% sensitivity [2, 3]. Histopathological examination remains the gold standard for detecting metastasis on resected LNs.

Various histological parameters associated nodal metastasis in OSCC have been studied by many authors out of which lymphovascular emboli (LVE), depth of invasion (DOI) and perineural invasion (PNI) are well-known. Other histological parameters that have been evaluated previously include degree of tumor histological differentiation (grade), host lymphocyte response (HLR), stromal response, and invasive tumor front (ITF). Pattern of tumor infiltration at the ITF including worst pattern of invasion (WPOI) and tumor budding (TB) are important pathological parameters in prediction of nodal metastasis in all stages of OSCC and associated with a poorer outcome in early-stage tumors [4]. Cancer cells located in the ITF have been suggested to be more aggressive in terms of metastatic potential and influence prognosis [5]. Tumors infiltrating in a widely dispersed manner are thought to be more aggressive than those infiltrating in a broad pushing fashion [6]. However, only WPOI-5 is mentioned in AJCC 8th edition. WPOI −4 and TB are not mentioned as essential criteria for reporting of OSCC.

Therefore, the aim of our study was to evaluate the role of POI, Tumor budding and other clinico-pathological parameters in determining the risk of nodal metastases in OSCC.

Materials and Methods

This was a retrospective study conducted in the departments of Pathology and Head and Neck Oncology at Rashtrasant Tukadoji Regional Cancer Centre, Nagpur, India. All the cases of OSCC who underwent surgical resection with cervical LN dissection as primary modality of treatment from Jan 2020 to May 2021 were included. Incomplete surgery, revision surgery, non-squamous pathology and where records were incomplete were excluded from the study. After retrieval of the slides, they were reviewed by a pathologist who was blinded to the clinical data.

The size of the tumor was noted from the surgical pathology report, and the pT stage was determined accordingly. The following histological parameters were evaluated in each case: grade, DOI, PNI, LVE, HLR, stromal response, pattern of invasion and tumor budding (TB).

The tumors were graded histologically as well, moderate and poorly differentiated according to their degree of differentiation. PNI was determined as a nerve being surrounded or infiltrated by the tumor. LVE was defined as tumor invasion within arterial, venous, or lymphatic vessels. HLR was evaluated at the invasive tumor front. HLR was graded semi-quantitatively as none/mild, moderate, and dense. For each case the slide with strongest HLR was selected. A dense HLR was assigned when diffuse continuous band-like lymphoid infiltrate with or without lymphoid aggregate was present at the interface. Only a few lymphocytes at the tumor interface were graded as mild HLR. Dense but discontinuous lymphoid infiltrate was graded as moderate HLR. The stromal response was graded as desmoplastic, loose, and minimal. The depth of invasion was measured in millimetres using slide calliper as the distance between the basement membrane of the adjacent unremarkable mucosa and the deepest part of the tumor. It was graded as D1 (≤ 5 mm), D2 (>5 to ≤ 10 mm), and D3 (>10 mm).

The invasive tumor front (ITF) was evaluated for pattern of invasion (POI) and tumor budding (TB). Invasive tumor front was defined as the deepest three to six cell layers or the detached tumor cell groups seen at the advancing edge of the tumor. Pattern of invasion (POI) refers to the manner in which the tumor infiltrates the host tissue at the tumor/host interface. The POI was determined as described in the literature previously and classified as 5 patterns. Pattern 1 was defined as broad, pushing margin of tumor with a smooth outline. Pattern 2 was defined as broad, pushing finger-like projection. Pattern 3 represents invasive tumor islands with >15 cells per island. Pattern 4 represents invasive tumor islands with less than 15 cells per island. Pattern 5 was defined by the presence of tumor island outside the main tumor at a distance of >1 mm. When multiple patterns of invasion were seen in a case, the score was determined by the highest pattern present, even if present focally. Among these 5 patterns, POI 4 and 5 were classified as invasive pattern, whereas POI 1 to 3 as cohesive pattern. Tumor budding was defined as single cells or a cluster of <5 tumor cells present in the stroma at the invasive tumor front. After screening all tumor slides number of tumor buds in one high-power field (HPF, 40X) was counted at the area of maximum concentration. A cut off point of three buds (≥ 3) was used to divide cases into high intensity verses low intensity TB [4, 7, 8]. The tumor budding and LHR is shown in figure 1 and worst pattern of invasion is shown in figure 2.

Patients for whom follow-up details were available were further evaluated to look for any loco-regional recurrence and/or distant metastasis. The relationship between Disease-free survival and POI and tumor budding was studied. Disease-free survival (DFS) was defined as the time from

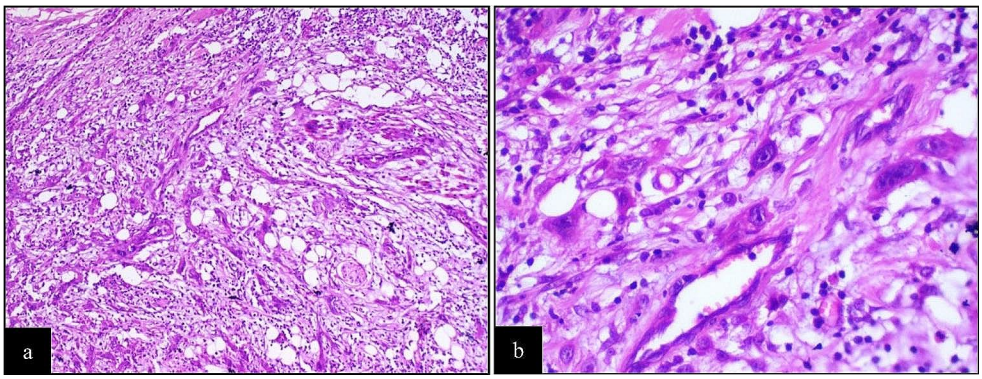


Fig. 1 (**a**) showing tumor budding & loose stroma and mild LHR on low power 4x (**b**) showing tumor budding and loose stroma on high power 40x

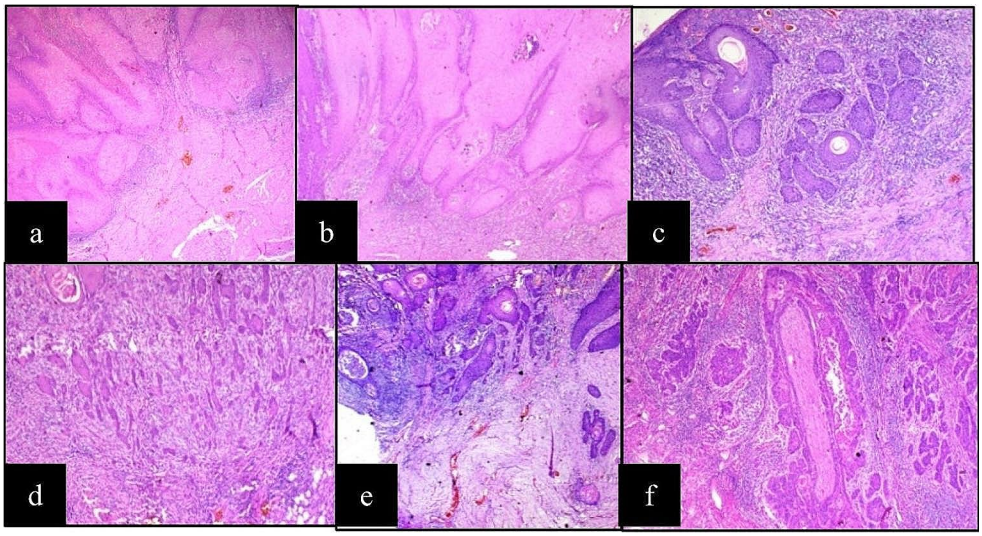


Fig. 2 (**a**) WPOI 1 (**b**) WPOI 2 (**c**) WPOI 3 (**d**) WPOI 4 (**e**) WPOI 5 (**f**) showing perineurial invasion and dense lymphocytic host response at the ITF

the date of surgery to the date of evidence of tumor recurrence at any site, death from any cause, or to the end of October 2023.

Statistical Analysis

Predictive significance of all the clinico-pathological parameters in nodal metastasis was assessed with Univariate analysis with the Fisher exact test and unpaired *t* test. The factors which were significant on univariate analysis were then analysed with multivariate analysis using a logistic regression model to find independent predictors. The significance was considered when $p < 0.05$. Disease-free survival analyses were performed using the Kaplan-Meier method and comparison done using the log-rank test for each group.

The statistical analysis was done using SPSS software for windows, version 23.0.

Results

Total 197 cases of OSCC were operated in our institute comprising of surgical resection and neck nodal dissection during the mentioned period. 107 cases were excluded from the study for not fitting into the inclusion criteria.

Distribution of demographic and clinico-pathological along with significant parameters predicting lymph node metastases on univariate analysis in all 90 cases is shown in Table 1.

Site, Grade, T stage, depth of invasion, perineurial invasion, lymphovascular emboli, high intensity tumor budding (TB ≥ 3/hpf), worst / infiltrative pattern of invasion and stroma all showed *p*- value < 0.05.

Factors predictive of nodal metastasis on multivariate analysis is shown in Table 2. Tumor budding, depth of invasion and perineural invasion showed a statistically significant result.

When calculated separately the nodal stage of the patients also showed significant correlation with average number of tumor buds as shown in Table 3.

The tumor buds were seen in 67 out of 90 cases (74.4%) while absent in 23 cases. TB ranged from 0 to 20 buds (mean

Table 1 Prognostic significance of clinical and histological parameters in relation to lymph node metastasis

No.	Parameters	No. of patients ($N=90$)	LN metastasis (+ve) ($n=42$); n (%)	LN metastasis (-ve) ($n=48$); n (%)	*P* Value
1.	Age (years)				0.401
	< 40	23	9 (10.0)	14(15.56)	
	> _40	67	33(36.66)	34(37.77)	
2.	Gender				0.297
	Male	73 (81.1)	36 (40.0)	37 (41.1)	
	Female	17 (18.9)	6 (6.67)	11 (12.22)	
3.	Site				0.031
	Buccal mucosa	45 (50)	20 (22.22)	25 (27.78)	
	Tongue	22 (24.4)	12 (13.33)	10 (11.11)	
	GBS	11 (12.2)	1 (1.1)	10 (11.11)	
	Alveolus	8 (8.9)	5 (5.56)	3 (3.33)	
	RMT	1 (1.1)	1 (1.11)	0 (0.0)	
	Lip	3 (3.3)	3 (3.33)	0 (0.0)	
4.	Grade				0.012
	Well	50 (55.6)	29 (32.22)	21 (23.33)	
	Moderate	34 (37.8)	13 (14.44)	21 (23.33)	
	Poor	6 (6.7)	0 (0.00)	6 (6.67)	
5.	T stage				< 0.001
	T1	7 (7.78)	7 (7.78)	0 (0.0)	
	T2	20 (22.2)	15 (16.67)	5 (5.56)	
	T3	33 (36.7)	8 (8.89)	25 (27.78)	
	T4	30 (33.3)	12 (13.33)	18 (20.00)	
6.	Depth of invasion				< 0.001
	D1	16 (17.78)	16 (17.78)	0 (0.0)	
	D2	30 (33.3)	15 (16.67)	15 (16.67)	
	D3	44 (48.9)	11 (12.22)	33 (36.67)	
7.	Perineural invasion				< 0.001
	Absent	57 (63.3)	36 (40.00)	21 (23.33)	
	Present	33 (36.7)	6 (6.67)	27 (30.00)	
8.	Lymphovascular invasion				0.018
	Absent	84 (93.3)	42 (46.67)	42 (46.67)	
	Present	6 (6.7)	0 (0.00)	6 (6.67)	
9.	Tumor budding				< 0.001
	Low	35	27 (30.00)	8 (8.89)	
	High (≥ 3)	55	15 (16.67)	40 (44.44)	
10.	Worst pattern of invasion				< 0.001
	Non-aggressive	20 (22.2)	17 (18.89)	3 (3.3)	
	Pattern 4	43 (47.8)	15 (16.67)	28 (31.11)	
	Pattern 5	27 (30.3)	10 (11.11)	17 (18.89)	
11.	Stroma				0.037
	Loose	37 (41.1)	16 (17.78)	31 (34.44)	
	Minimal	47 (52.2)	3 (3.33)	3 (3.33)	
	Desmoplastic	6 (6.7)	23 (25.56)	14 (15.56)	
12.	Host lymphocytic response				0.060
	Moderate	39 (43.3)	15 (16.67)	23 (25.56)	
	Dense	38 (42.2)	24 (26.67)	15 (16.67)	
	Mild	12	3 (3.33)	9 (10.00)	
	No	1	0 (0.00)	1 (1.11)	
13.	Skin				0.534
	Involved	10 (11.1)	4(4.44)	6 (6.67)	
	Not involved	57 (63.3)	25 (27.78)	32 (35.56)	
	Not applicable	23 (25.6)	13 (14.44)	10 (11.11)	
14.	Bone				0.527
	Involved	22 (24.4)	9 (10.0)	13 (14.44)	
	Not involved	45 (50.0)	20 (22.22)	25 (27.78)	
	Not applicable	23 (25.6)	13 (14.44)	10 (11.11)	

Table 2 Multivariate logistic regression analysis for parameters predicting nodal metastasis in OSCC

Parameters	B	SE	OR	95% CI	*P* Value
POI	0.023	0.570	1.024	0.335–3.130	0.967
TB	2.006	0.832	7.430	1.455–37.951	0.016
Stroma	-0.301	0.350	0.740	0373-1.469	0.390
Site	0.672	0.386	1.958	0.918–4.173	0.082
Grade	-0.517	0.367	0.597	0.290–1.225	0.160
DOI	1.572	0.653	4.818	1.341–17.311	0.016
T stage	-0.120	0.485	0.887	0.343–2.295	0.805

Table 3 Relationship of number of tumor buds and lymph node stage

Nodal stage	No. of cases	Average no. of TB	*P* Value
N0	42	2.02	< 0.001
NI	13	4	
N2A	2	14.5	
N2B	22	3.95	
N3B	11	4.64	

4.54). Of the 67 cases high intensity (≥ 3 buds/hpf) TB was seen in 55 cases, rest of the cases show ≤ 2 buds/hpf. Association of TB with other clinico-pathological parameters is shown in Table 4. Statistically significant association of high intensity TB was noted with grade ($P = 0.001$), depth of invasion ($P = 0.007$), perineural invasion (P = < 0.001), nodal metastasis (P = < 0.001), aggressive POI (pattern 4 followed by pattern 5) (P = < 0.001), tumor stroma ($P = 0.001$) and host lymphocyte response ($P = 0.027$). Intensity of TB was associated with increased risk of nodal metastasis where, 72.7% of the cases with high intensity tumor budding showed nodal metastasis compared with only 22.9% of those with low intensity budding. Higher T stage, skin and bone involvement were more commonly associated with high intensity budding, however the association was not statistically significant ($P > 0.05$).

Association of pattern of invasion with other clinico-pathological variables is shown in Table 5. Aggressive pattern of invasion, especially pattern 4 was significantly associated with tumor budding ($P = 0.037$). High incidence of nodal metastasis with statistical significance was seen in cases with WPOI (P = < 0.001). A statistically significant association of aggressive POI was also found with male gender ($P = 0.014$), grade ($P = 0.011$), depth of invasion ($P = 0.004$), perineurial invasion ($P = 0.000$), loose tumor stroma ($P = 0.001$).

Out of total 90 cases follow-up information was available in only 73 cases. The rest of the patients were lost to follow-up. The average duration of follow-up was 14 months (range 6 to 36 months). During this period 16 patients had local recurrence, one patient developed distant metastasis and one patient died due to another cause not related to the disease.

As shown in plot 1, a statistically significant difference in 3-year DFS was observed among patients with infiltrative POI (pattern 4 and 5 together) verses non-infiltrative POI (pattern1-3) (P value = 0.0372). We did not find a statistically significant association after separating pattern 4 and 5. The intensity of tumor budding was also a prognostic parameter for DFS (P value = 0.0489).

Discussion

Lymph nodal metastasis is one of the most important independent adverse prognostic factors in OSCC influencing patient survival and multimodality treatment decisions including radiotherapy. The reported occult metastases rate is about 26% in all stages of buccal cancer [9]. Hence it is crucial to identify the risk factors predicting nodal metastasis. The intention of our study was to build upon the previous studies about role of known clinico-pathologic variables with special emphasis on emerging factors like TB, WPOI, and LHR at the ITF influencing nodal metastasis and thus further prognosis. Invasive tumor front is an area of current research interest providing most useful prognostic information [10].

In accordance to previous studies we found that presence of TB serves as independent prognosticator affecting LN metastasis [4, 11]. TB is reveals loss of cell cohesion, epithelial mesenchymal transition and active invasive movement of cancer cells playing major role in initiation of metastasis [12]. TB is associated with increased biologic aggressiveness, locoregional recurrence, distant metastasis and decreased survival. It is an inexpensive, readily identifiable and easy histological parameter with less interobserver variability [11]. Many previous studies have used a cut off of ≥ 5 buds on 20x objective for association with nodal metastasis [8]. We used a cut off of ≥ 3 buds on 40x objective which is universally present on all microscopes like a study by Chatterjee et al. and found statistically significant association of high intensity TB with LN metastasis on both univariate and multivariate analysis [4]. In few studies TB was evaluated in preoperative biopsies to predict risk of LN metastasis in OSCC with high sensitivity [7, 13].

In our study, TB was seen to be associated significantly with other clinico-pathological variables like DOI, grade, WPOI, LHR, stroma, perineural invasion. Angadi et al. found association between TB and DOI [14]. Significant association between TB and DOI, PNI, LVI, T stage, POI, grade was noted in previous study [15].

Multiple studies have shown WPOI as independent predictor of LN metastasis [4, 6, 8, 14, 16, 17]. Contrarily, Lundqvist et al. and Kane et al. didn't find significant association between WPOI and nodal metastasis [18, 19]. We

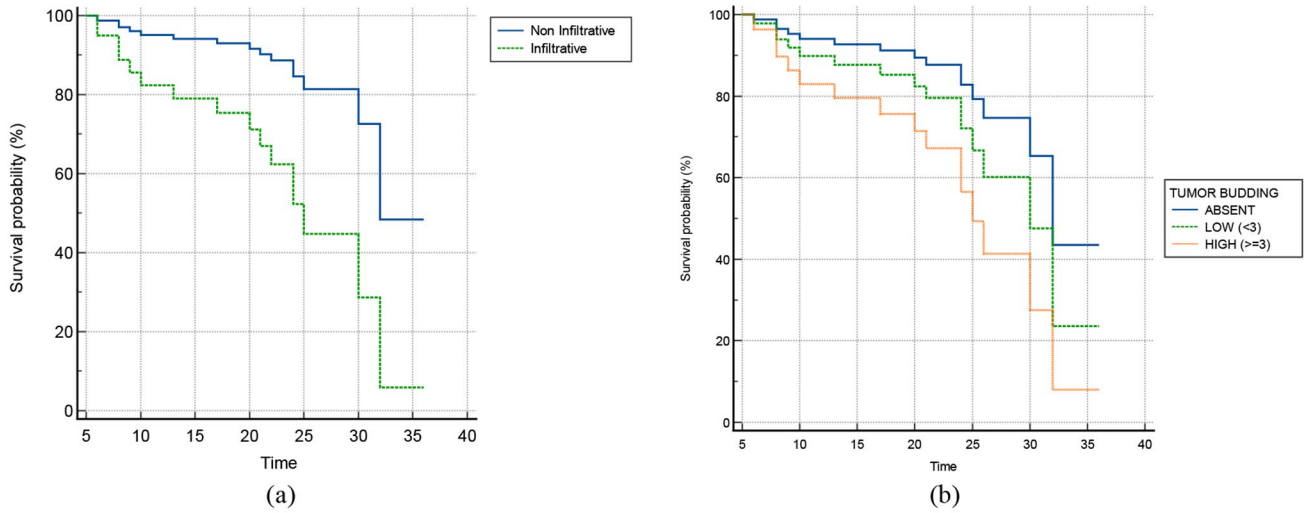
Table 4 TB intensity correlated with clinico-pathological parameters

No.	Parameters	Low intensity (< 3 buds/hpf) ($n = 35$); n (%)	High intensity (≥ 3 buds/hpf) ($n = 55$); n (%)	*P* Value
1.	Gender			0.046
	Male	32 (35.6)	41 (45.6)	
	Female	3 (3.3)	14 (15.6)	
2.	Site			0.077
	Buccal mucosa	19 (21.1)	26 (28.9)	
	Tongue	5 (5.6)	17 (18.9)	
	GBS	3 (3.3)	8 (8.9)	
	Alveolus	4 (4.4)	4 (4.4)	
	RMT	1 (1.11)	0 (0.0)	
	Lip	3 (3.3)	0 (0.0)	
3.	Grade			0.001
	Well	28 (31.1)	22 (24.4)	
	Moderate	6 (6.7)	28 (31.1)	
	Poor	1 (1.1)	5 (5.6)	
4.	T stage			0.376
	T1	4 (4.4)	3 (3.3)	
	T2	10 (11.1)	10 (11.1)	
	T3	10 (11.1)	23 (25.6)	
	T4	11 (12.2)	19 (21.1)	
5.	Depth of invasion			0.007
	D1	11 (12.22)	5 (5.6)	
	D2	13 (14.4)	17 (18.9)	
	D3	11 (12.22)	33 (36.67)	
6.	Perineural invasion			< 0.001
	Absent	31 (34.4)	26 (28.9)	
	Present	4 (4.4)	29 (32.2)	
7.	Lymphovascular invasion			0.773
	Absent	33 (36.7)	51 (56.7)	
	Present	2 (2.2)	4 (4.4)	
8.	Lymph node			< 0.001
	Positive	27 (30.00)	15 (16.7)	
	Negative	8 (8.9)	40 (44.44)	
9.	Worst pattern of invasion			< 0.001
	Non-aggressive	20 (22.22)	0 (0.0)	
	Pattern 4	11 (12.2)	32 (35.6)	
	Pattern 5	4 (4.4)	23 (25.6)	
10.	Stroma			0.001
	Loose	10 (11.1)	37 (41.1)	
	Minimal	4 (4.4)	2 (2.2)	
	Desmoplastic	21 (23.3)	16 (17.8)	
11.	Host lymphocytic response			0.027
	Moderate	10 (11.1)	28 (31.1)	
	Dense	22 (24.4)	17 (18.9)	
	Mild	3 (3.3)	9 (10.0)	
	No	0 (0.0)	1 (1.1)	
12	Skin			0.090
	Involved	27 (30.0)	30 (33.33)	
	Not involved	2 (2.2)	8 (8.88)	
	Not applicable	6 (6.7)	17 (18.88)	
13.	Bone			0.334
	Involved	19 (21.11)	26 (28.88)	
	Not involved	10 (11.1)	12 (13.33)	
	Not applicable	6 (6.7)	17 (18.88)	

Table 5 Correlation of pattern of invasion with clinico-pathological parameters

No.	Parameters	Non-aggressive pattern	Pattern 4	Pattern 5	*P* Value
1.	Gender				0.014
	Male	20 (22.22)	30 (33.33)	23 (25.55)	
	Female	0 (0.0)	13 (14.44)	4 (4.4)	
2.	Site				0.479
	Buccal mucosa	12 (13.33)	19 (21.11)	14 (15.56)	
	Tongue	3 (3.33)	11 (12.22)	8 (8.88)	
	GBS	2 (2.22)	6 (6.67)	3 (3.33)	
	Alveolus	1 (1.11)	6 (6.67)	1 (1.11)	
	RMT	0 (0.0)	1 (1.11)	0 (0.00)	
	Lip	2 (2.22)	0 (0.0)	1 (1.11)	
3.	Grade				0.011
	Well	18 (20.00)	21 (23.33)	11 (12.22)	
	Moderate	2 (2.22)	19 (21.1)	3 (3.33)	
	Poor	0 (0.0)	3 (3.33)	13 (14.44)	
4.	T stage				0.511
	T1	2 (2.22)	3 (3.33)	2 (2.22)	
	T2	7 (7.77)	6 (6.67)	7 (7.77)	
	T3	7 (7.77)	26 (17.77)	10 (11.1)	
	T4	4 (4.44)	18 (20.00)	8 (8.88)	
5.	Depth of invasion				0.004
	D1	9 (10.00)	4 (4.44)	3 (3.33)	
	D2	7 (7.77)	14 (15.56)	9 (10.00)	
	D3	4 (4.44)	25 (27.78)	15 (16.67)	
6.	Perineural invasion				< 0.001
	Absent	18 (20.00)	27 (30.00)	12 (13.33)	
	Present	2 (2.22)	16 (17.77)	15 (16.66)	
7.	Lymphovascular invasion				0.942
	Absent	19 (21.11)	40 (44.44)	25 (27.78)	
	Present	1 (1.11)	3 (3.33)	2 (2.22)	
8.	Lymph node				< 0.001
	Positive	17 (18.88)	15 (16.7)	10 (11.11)	
	Negative	3 (3.33)	28 (31.11)	17 (18.88)	
9.	Tumor budding				0.037
	Absent	20 (22.22)	0 (0.0)	3 (3.33)	
	Present	0 (0.00)	43 (47.77)	24 (26.66)	
10.	Stroma				0.001
	Loose	5 (5.56)	24 (26.66)	18 (20.00)	
	Minimal	3 (3.33)	3 (3.33)	0 (0.00)	
	Desmoplastic	12 (13.33)	16 (17.78)	9 (10.00)	
11.	Host lymphocytic response				0.125
	Moderate	5 (5.55)	21 (23.33)	12 (13.33)	
	Dense	14 (15.56)	16 (17.78)	9 (10.00)	
	Mild	1 (1.11)	5 (5.56)	6 (6.67)	
	No	0 (0.0)	1 (1.11)	0 (0.00)	
12	Skin				0.345
	Involved	0 (0.00)	6 (6.67)	4 (4.44)	
	Not involved	16 (17.78)	26 (28.88)	15 (15.56)	
	Not applicable	4 (4.44)	11 (12.22)	8 (8.88)	
13.	Bone				0.687
	Involved	4 (4.44)	13 (14.44)	5 (5.56)	
	Not involved	12 (13.33)	19 (21.11)	14 (15.56)	
	Not applicable	4 (4.44)	11 (12.22)	8 (8.88)	

found highly significant association between worst pattern of invasion (pattern 4 and 5) and LN metastasis on univariate analysis (P = < 0.0001); which was not found significant on multivariate analysis. Like our study, Sakata et al. found that TB but not WPOI as independent marker on multivariate analysis [20]. We also studied association of aggressive POI with other clinico-pathologic parameters and found it significant with male gender, grade, DOI, PNI, and loose



Plot 1 Plots of survival probability vs. time since treatment (in months) for different. (**a**) Infiltrative and non-infiltrative POI (**b**) Tumor budding absent and present

tumor stroma. Mishra et al. also discovered a significant association of non-cohesive pattern with T stage, DOI, PNI and grade [21].

In our study, DOI was one of the most significant risk factors in both univariate and multivariate analysis. In patients of early OSCC, DOI ≥ 5 mm is associated with increased risk of nodal metastasis and should be considered for elective neck node dissection [19]. In previous literature a cut off of DOI ≥ 4 mm was considered as an indicator of poor prognosis with increased risk of nodal metastasis, however, many recent studies found 5 mm as most useful cut off for early OSCC [6, 22].

LVE and PNI are important risk factors for cervical node metastasis in OSCC in all stages should be included as mandatory part in reporting format [4, 16]. Martinez-Gimeno et al. found a metastatic rate of 54.5% in cases with PNI compared to only 32.8% in those without PNI [23]. Aditi el al stated that the presence of PNI independently predicts LN metastasis with 85% sensitivity and 83% specificity [16]. We also found PNI as independent predictor of LN metastasis with LN metastasis of 81.8% in cases with PNI Vs 36.8% in cases without PNI.

LVE is significantly associated with survival, grade, pattern of invasion, LN metastasis and local recurrence [24]. Martinez-Gimeno et al. reported metastasis in 74.2% patients with LVE as compared to only 2.1% in patients without LVE [23]. In our study, LVE showed significant association with LN metastasis ($P = 0.018$) with a metastasis rate of 100% in cases with LVE as compared to 50% in those without LVE, consistent with the results of previous studies.

Histologic grade of tumor and degree of lymphoid response at ITF are independent predictors of cervical nodal metastasis in OSCC [16, 25]. There are many studies proving effect of tumor grade on cervical LN metastasis [23, 26]. In present study, we found a significant correlation between tumor grade and lymphoid response on univariate analysis which could not be proved on multivariate analysis. This could be because of limited sample size. Kane et al. in a study of 48 cases of early OSCC did not report any correlation between tumor grade and LHR with cervical nodal metastasis [19]. Martinez-Gimeno et al. also did not find a statistically significant association with lymphoid response.

Similar to our study Parekh et al. showed that WPOI-4 and -5 taken together significantly affected DFS ($P = 0.035$) as compared with patterns 1–3 ($P = 0.035$), whereas pattern 5 alone did not ($P = 0.910$). They found significant difference in 3-year DFS of patients with ≥ 3 buds verses those with 0–2 buds, (P value = 0.021) [27]. A meta-analysis by Almangush et al. showed a significant correlation between the presence of > 5 TB and poor disease-free survival [11]. Shimizu et al. found that WPOI, and tumor budding were significant predictors of 5-year disease-free survival ($p = 0.03$, and $p < 0.01$) respectively [28].

Conclusion

In the present study, we found a significant association of lymph node metastasis with various clinico-pathological variables like tumor budding, depth of invasion, perineural invasion (on multivariate analysis) and worst pattern of invasion type 4 and 5, stroma, lymphocyte host response, lymphovascular emboli, T stage and site (on univariate analysis). Among these variables, role of LVI, PNI, DOI are already well established. The emerging significant

parameters are TB, WPOI and LHR. They are easy to report on haematoxylin and eosin stained sections itself with good interobserver agreement. We found it significantly affects the nodal status and thus further prognosis and management. Hence, based on the findings of the present study and the review of previous studies, these parameters should be included in routine reporting format of OSCC. There is a future need of detailed studies on tumor budding and reporting it especially on pre-operative biopsies so that patients with biological aggressive tumor with higher risk of nodal metastasis can be identified. Limitation of the study is a small sample size and short duration of follow-up.

Author Contributions The manuscript has been reads and approved by all the authors and each author satisfies the authorship requirements. Dr. Renuka Badanale- Conception and design of study, Acquisition of data, Analysis and interpretation of data, Drafting the manuscript. Dr. Vandana Mohta- Conception and design of study, Acquisition of data, Analysis and interpretation of data, Critical revision. Dr. Aniruddha Wagh- Acquisition of data, Critical revision. Dr. Kartar Singh- Acquisition of data, Critical revision.

Data Availability Yes.

Declarations

Ethics Approval Ethical approval was waived by the local ethics committee in view of retrospective nature of the study and all the procedures being performed were part of routine care.

Consent NA.

Sources of Support NIL.

Financial Disclosure No sources of funding.

Conflict of Interest None.

References

1. Massano J, Regateiro FS, Januário G, Ferreira A (2006) Oral squamous cell carcinoma: review of prognostic and predictive factors. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontology. ;102(1):67–76
2. Lim S-C, Zhang S, Ishii G, Endoh Y, Kodama K, Miyamoto S et al (2004) Predictive markers for late cervical metastasis in stage I and II invasive squamous cell carcinoma of the oral tongue. Clin Cancer Res 10(1):166–172
3. Khafif RA, Gelbfish GA, Tepper P, Attie JN (1991) Elective radical neck dissection in epidermoid cancer of the head and neck. A retrospective analysis of 853 cases of mouth, pharynx, and larynx cancer. Cancer 67(1):67–71
4. Chatterjee D, Bansal V, Malik V, Bhagat R, Punia RS, Handa U et al (2019) Tumor budding and worse pattern of invasion can predict nodal metastasis in oral cancers and associated with poor survival in early-stage tumors. Ear Nose Throat J 98(7):E112–E9
5. Bankfalvi A, Piffko J (2000) Prognostic and predictive factors in oral cancer: the role of the invasive tumour front. J Oral Pathol Medicine: Rev Article 29(7):291–298
6. Manjula B, Augustine S, Selvam S, Mohan AM (2015) Prognostic and predictive factors in gingivo buccal complex squamous cell carcinoma: role of tumor budding and pattern of invasion. Indian J Otolaryngol Head Neck Surg 67:98–104
7. Seki M, Sano T, Yokoo S, Oyama T (2016) Histologic assessment of tumor budding in preoperative biopsies to predict nodal metastasis in squamous cell carcinoma of the tongue and floor of the mouth. Head Neck 38(S1):E1582–E90
8. Hori Y, Kubota A, Yokose T, Furukawa M, Matsushita T, Oridate N (2020) Association between pathological invasion patterns and late lymph node metastases in patients with surgically treated clinical no early oral tongue carcinoma. Head Neck 42(2):238–243
9. Diaz EM Jr, Holsinger FC, Zuniga ER, Roberts DB, Sorensen DM (2003) Squamous cell carcinoma of the buccal mucosa: one institution's experience with 119 previously untreated patients. Head Neck: J Sci Specialties Head Neck 25(4):267–273
10. Bryne M, Koppang HS, Lilleng R, Kjærheim Å (1992) Malignancy grading of the deep invasive margins of oral squamous cell carcinomas has high prognostic value. J Pathol 166(4):375–381
11. Almangush A, Pirinen M, Heikkinen I, Mäkitie AA, Salo T, Leivo I (2018) Tumour budding in oral squamous cell carcinoma: a meta-analysis. Br J Cancer 118(4):577–586
12. Mäkitie AA, Almangush A, Rodrigo JP, Ferlito A, Leivo I (2019) Hallmarks of cancer: Tumor budding as a sign of invasion and metastasis in head and neck cancer. Head Neck 41(10):3712–3718
13. Seki M, Sano T, Yokoo S, Oyama T (2017) Tumour budding evaluated in biopsy specimens is a useful predictor of prognosis in patients with cN0 early stage oral squamous cell carcinoma. Histopathology 70(6):869–879
14. Angadi PV, Patil PV, Hallikeri K, Mallapur M, Hallikerimath S, Kale AD (2015) Tumor budding is an independent prognostic factor for prediction of lymph node metastasis in oral squamous cell carcinoma. Int J Surg Pathol 23(2):102–110
15. Ho YY, Wu TY, Cheng HC, Yang CC, Wu CH (2019) The significance of tumor budding in oral cancer survival and its relevance to the eighth edition of the American Joint Committee on Cancer staging system. Head Neck 41(9):2991–3001
16. Arora A, Husain N, Bansal A, Neyaz A, Jaiswal R, Jain K et al (2017) Development of a new outcome prediction model in early-stage squamous cell carcinoma of the oral cavity based on histopathologic parameters with multivariate analysis. Am J Surg Pathol 41(7):950–960
17. Khwaja T, Tayaar AS, Acharya S, Bhushan J, Muddapur M (2018) Pattern of invasion as a factor in determining lymph node metastasis in oral squamous cell carcinoma. J Cancer Res Ther 14(2):382–387
18. Lundqvist L, Stenlund H, Laurell G, Nylander K (2012) The importance of stromal inflammation in squamous cell carcinoma of the tongue. J oral Pathol Med 41(5):379–383
19. Kane S, Gupta M, Kakade A, D'Cruz A (2006) Depth of invasion is the most significant histological predictor of subclinical cervical lymph node metastasis in early squamous carcinomas of the oral cavity. Eur J Surg Oncol (EJSO) 32(7):795–803
20. Sakata J, Yamana K, Yoshida R, Matsuoka Y, Kawahara K, Arita H et al (2018) Tumor budding as a novel predictor of occult metastasis in cT2N0 tongue squamous cell carcinoma. Hum Pathol 76:1–8
21. Mishra A, Das A, Dhal I, Shankar R, Bhavya B, Singh N et al (2022) Worst pattern of invasion in oral squamous cell carcinoma is an independent prognostic factor. J Oral Biology Craniofac Res 12(6):771–776
22. Huang SH, Hwang D, Lockwood G, Goldstein DP, O'Sullivan B (2009) Predictive value of tumor thickness for cervical lymph-node involvement in squamous cell carcinoma of the oral cavity: a meta-analysis of reported studies. Cancer: Interdisciplinary Int J Am Cancer Soc 115(7):1489–1497

23. Vila CN, Martínez-Gimeno C, Rodríguez EM, Varela CL (1995) Squamous cell carcinoma of the oral cavity: a clinicopathologic scoring system for evaluating risk of cervical lymph node metastasis. Laryngoscope 105(7):728–733
24. Woolgar JA, Scott J (1995) Prediction of cervical lymph node metastasis in squamous cell carcinoma of the tongue/floor of mouth. Head Neck 17(6):463–472
25. Hiratsuka H, Miyakawa A, Nakamori K, Kido Y, Sunakawa H, Kohama G (1997) Multivariate analysis of occult lymph node metastasis as a prognostic indicator for patients with squamous cell carcinoma of the oral cavity. Cancer: Interdisciplinary Int J Am Cancer Soc 80(3):351–356
26. Borges A, Shrikhande S, Ganesh B (eds) (1989) Surgical pathology of squamous carcinoma of the oral cavity: its impact on management. Seminars in Surgical Oncology. Wiley Online Library
27. Parekh D, Kukreja P, Mallick I, Roy P (2020) Worst pattern of invasion–type 4 (WPOI-4) and Lymphocyte host response should be mandatory reporting criteria for oral cavity squamous cell carcinoma: a re-look at the American Joint Committee of Cancer (AJCC) minimum dataset. Indian J Pathol Microbiol 63(4):527
28. Shimizu S, Miyazaki A, Sonoda T, Koike K, Ogi K, Kobayashi J-i et al (2018) Tumor budding is an independent prognostic marker in early stage oral squamous cell carcinoma: with special reference to the mode of invasion and worst pattern of invasion. PLoS ONE 13(4):e0195451