Indian Journal of Otolaryngology and Head & Neck Surgery
https://doi.org/10.1007/s12070-024-05050-7

ORIGINAL ARTICLE

# Neoteric Predictors for Lymph Node Metastasis in Early Oral Squamous Cell Carcinoma: Tumor Budding and Worst Pattern of Invasion

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Received: 17 February 2024 / Accepted: 22 August 2024


## Abstract

Oral cancer is one of the most common cancers seen in the Indian subcontinent. Its primary treatment is surgery with or without adjuvant treatment. Despite advances in science, prognosis and overall survival has not yet chanced over the past two decades. Pathologically proven regional lymph node metastasis adversely affects the prognosis. This study was conducted to evaluate the predictive factors for lymph node metastasis in Stage I and II oral squamous cell carcinoma (OSCC) with distinct emphasis on tumor budding and worst pattern of invasion. This is a prospective observational study was done at a tertiary care center, Prince Aly Khan Hospital, Mumbai, over a period of 22 months (March, 2020 to December, 2021). We analyzed 237 patients of early OSCC for clinicopathological parameters (age, trismus, differentiation, depth of invasion, tumor budding, worst pattern of invasion). Chi Square test and logistic regression model were used for data evaluation. Statistical Package for Social Sciences, version 21.0 IBM Corporation USA for Microsoft Windows, was used for data analysis. This study reported statistically significant predictive factors for lymph node metastasis viz. tumor budding (OR 30.8 95% CI 12.365–76.731 $p<0.001$), worst pattern of invasion (OR 4.5 95% CI 1.853–11.305 $p=0.001$) and age (OR 0.149 95% CI 0.043–0.0516 $p<0.003$) on logistic regression model. On Chi square test, along with the above factors- tumor differentiation ($p=0.008$) and depth of invasion ($p=0.001$) were also found statistically significant in prediction for lymph node metastasis in early OSCC. Strong predictive association exists between lymph node metastasis and tumor budding, worst pattern of invasion and higher age group in early OSCC. These factors can be adapted as a routinely assessed predictive marker and mentioned in histopathology reports with its prognostic implications, thus can be considered for further planning and management. These predictive factors can be used to formulate a risk score to incorporate various clinicopathological factors including tumor budding, worst pattern of invasion, depth of invasion, tumor differentiation and T stage which can be used in patients diagnosed with early stage I & II OSCC where neck dissection can be avoided.

**Keywords** Oral cancer · WPOI · Tumour budding · Prognosis

## Introduction

Oral cancer is the most common malignancy of the head and neck region, globally with an annual incidence 377,713 cases and mortality 177,757 cases (*GLOBOCAN 2020)*. India has one third of oral cancer cases in the world. It is the 2nd top frequent cancer of India [1]. The early-stage oral cancer includes T1-T2, N0, M0 show a favorable outcome, however prognosis is variegated and depends upon multiple clinicopathological parameters. The established factors include stage, lymph node metastasis, perineural invasion (PNI) and extra nodal extension (ENE) [2]. Several studies have indicated that involvement of cervical lymph node

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Published online: 17 September 2024

in OSCC patients decreases significantly survival rate up to ~50% than those with disease free nodes.

Neoteric histological parameters are the presence of tumor budding (TB) and worst pattern of invasion (WPOI). These parameters have shown to be associated with high incidence of lymph node metastasis and relapse in colorectal, esophagus, endometrial and lung cancers [3–5]. A few previous studies have evaluated the role of TB and WPOI in oral cavity cancers and found its association with higher incidence of nodal metastasis, recurrence and poor overall survival.

Histological predictive and prognostic factors have been infrequently evaluated in Indian patients. This study is conducted, in a tertiary cancer care hospital, to evaluate the role of histological parameters with distinct emphasis on TB and WPOI to predict the risk of nodal metastasis in cases of OSCC in Indian subcontinent which translates to the role of elective versus therapeutic neck dissection in early OSCC and prognosis.

## Methods

### Study Design

All patients coming to Prince Aly Khan Hospital, Mumbai with previously untreated OSCC with clinical stage I and II were included in this study. The study duration of this prospective observational study was 22 months between March, 2020 to December, 2021. Sample size was calculated using proportions of relative precision and estimated sample size is 237 patients. Following predictive factors were considered under study- clinical factors viz. age, stage, oral submucous fibrosis (OSMF), trismus; histopathological factors viz. tumor size, pathological lymph node status, depth of invasion (DOI), differentiation grade, perineural invasion (PNI), lymphovascular invasion (LVI), WPOI and TB. Informed consent was taken from all the patients meeting inclusion criteria. All the early stage OSCC were managed with wide local excision and neck dissection. The defect was closed per primum or using local/ regional flaps. The diagnostic tissue blocks were retrieved from pathology department and used in this study. The tumor extent and the histopathological assessment were classified according to the eighth edition of the AJCC TNM Classification [6]. The study was conducted after obtaining permission from ethics committee of the hospital.

### Definitions

Tumor budding (TB) was defined as the presence of isolated single tumor cells or a small group of fewer than five cells ahead of the deep invasive tumor front. Immunostained slides were initially studied with a 4x objective lens to select the areas with the highest density of budding, called hotspot. TB at the hotspot was then counted using a 20x objective lens, and the highest count per slide was used as the number of buds. The intensity of TB was categorized into low intensity (0–4 buds/field) intermediate (5–9 buds/field) and high intensity (>=10buds/field) according to the ITBCC 2016 recommendations [7]. In this study TB was categorized into two groups: - Group 1-low (less than 5 buds/ field) (Fig. 1a) and Group 2- high (equal or more than 5 buds/ field). (Fig. 1b)

The invasive tumor front was evaluated for pattern of invasion (POI). The POI was determined as described in the literature previously and classified as 5 patterns [8, 9] Type 1 have pushing borders, Type 2 have finger-like growth, Type 3 have large separate islands with more than 15 cells per island, Type 4 have small tumor islands with 15 cells or fewer per island and Type 5 have tumor satellites equal or more than 1 mm from main tumor or next closest satellites (Fig. 1c-f). In this study WPOI was categorized into two groups: Group 1 WPOI 1–3 and Group 2 WPOI 4–5.

### Tumor Sample Preparation

The slides were made from the formalin fixed paraffin embedded and stained with H&E. Anti-cytokeratin Cocktail (AE1 & AE3) was used as the primary antibody for IHC staining. The advantage of using cytokeratin include shorter working time, lower difficulty, greater replicability and it has produced significant better reproducibility as well as inter and intra-observer agreement as compared to H&E.

In evaluating each microscopic feature, special attention was paid to peripheral areas of the most invasive carcinoma stack. If different grades of each criterion were observed in the same specimen, the higher grade was taken as representative. These invasive tumor patterns were assessed on Olympus Magnus MX21i microscope by two authors simultaneously and confirmed with senior Head and neck oncopathologist.

### Statistical Analysis

The data on categorical variables is shown as n (% of cases) and data on continuous variables is shown as mean ± standard deviation (SD). The inter-group statistical significance of difference of categorical variables is tested using Chi- Square test and logistic regression analysis was used to predict the association between the major independent variables and lymph node metastasis. In the entire study, the p-values less than 0.05 are considered to be statistically significant. Statistical Package for Social Sciences (SPSS

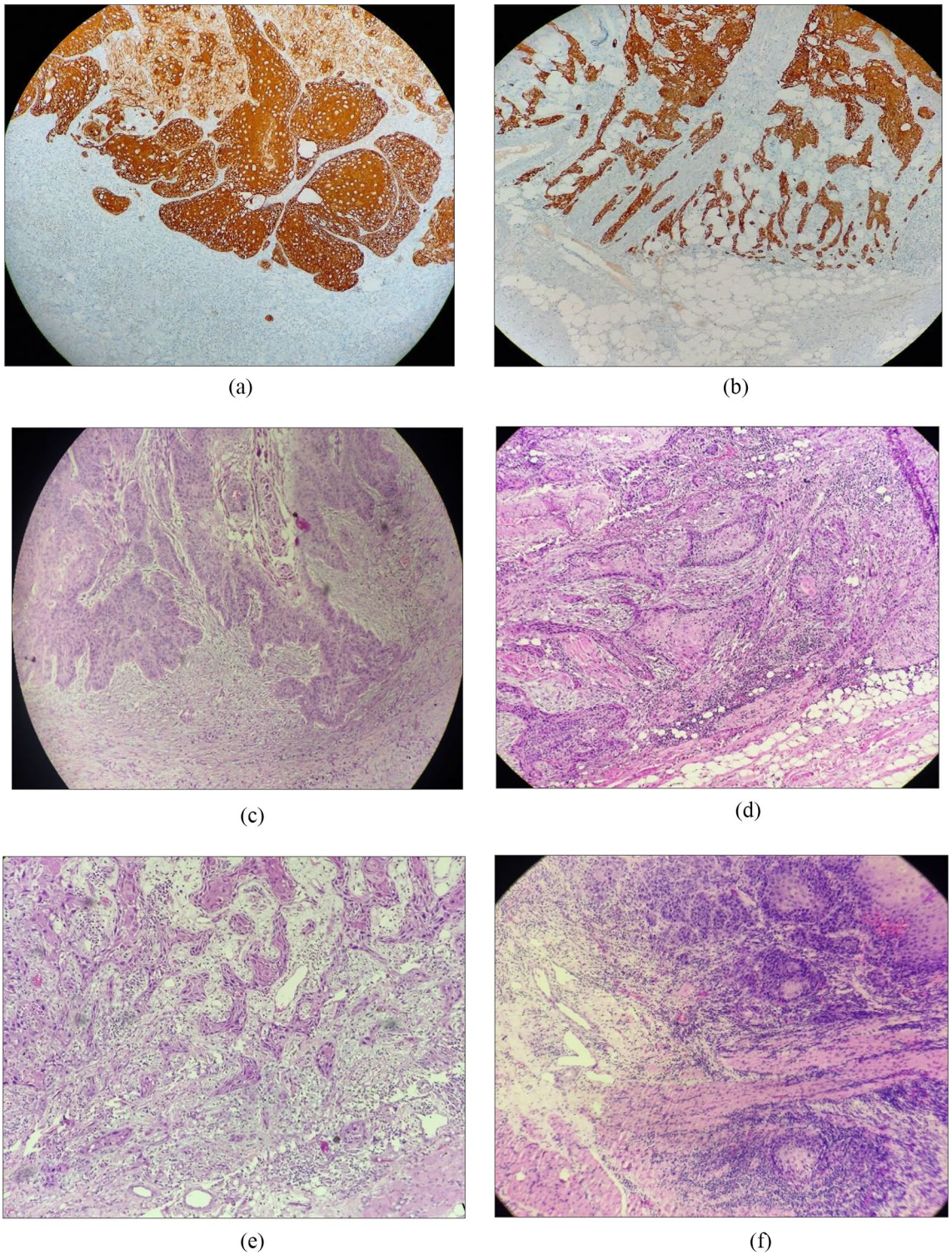


**Fig. 1** **(a)** Tumour Budding < 5: Low Buds **(b)** Tumour Budding ≥ 5: High Buds **(c)** Worst Pattern of invasion- Type 2 **(d)** Worst Pattern of invasion- Type 3 **(e)** Worst Pattern of invasion- Type 4 (**f** )Worst Pattern of invasion- Type 5

**Table 1** Association between tumor budding with various clinicopathological factors

| Variables | No. of Patients | Tumour Budding | | *p*-value |
|---|---|---|---|---|
| | | Low | High | |
| Sex | | | | 0.699 |
| Female | 25 | 17 (10.1%) | 8 (11.8%) | |
| Male | 212 | 152 (89.9%) | 60 (88.2%) | |
| Age | | | | **0.022** |
| 25–40 years | 62 | 10 (14.7%) | 52 (30.8%) | |
| 41–60 years | 132 | 41 (60.3%) | 91 (53.8%) | |
| 61 + years | 43 | 17 (25%) | 26 (15.4%) | |
| Clinical T stage | | | | 0.07 |
| cT1 | 69 | 55 (32.5%) | 14 (20.6%) | |
| cT2 | 168 | 114 (67.5%) | 54 (79.4%) | |
| Depth of invasion | | | | **0.003** |
| </= 6 mm | 140 | 110 (65.1%) | 30 (44.1%) | |
| > 6 mm | 97 | 59 (34%) | 38 (55.9%) | |
| Perineural invasion | | | | 0.13 |
| Absence | 48 | 134 (79.3%) | 48 (70.6%) | |
| Presence | 20 | 35 (20.7%) | 20 (29.4%) | |
| Histologic grade | | | | **0.006** |
| Well differentiated | 29 | 28 (16.6%) | 1 (1.5%) | |
| Moderately differentiated | 164 | 111 (65.7%) | 53 (77.9%) | |
| Poorly differentiated | 44 | 30 (17.8%) | 14 (20.6%) | |
| Worst pattern of invasion (WPOI) | | | | **< 0.001** |
| WPOI-1,2,3 | 111 | 92 (54.4%) | 19 (27.9%) | |
| WPOI-4 & 5 | 126 | 77 (45.6%) | 49 (72.1%) | |
| Tobacco Consumption | | | | 0.273 |
| No | 55 | 36 (21.3%) | 19 (27.9%) | |
| Yes | 182 | 133 (78.7%) | 49 (72.1%) | |

version 21.0 IBM Corporation, USA) for Microsoft Windows was used for data analysis.

## Results

A total of 237 patients were enrolled in this study between March, 2020 to December, 2021 and the results are as follows:

### Descriptive Statistics

The mean age of the patients was 48.03 ± 11.32 years with a range of 28 to 81 years and 212 (89.5%) were male, while 25 (10.5%) were female. Oral submucous fibrosis (OSMF) is a potentially malignant condition caused by the use of areca nut and 144(60.8%) patients had OSMF. In this study 53(22.4%) had trismus while 184(77.6%) had normal mouth opening. None of the patients had lymphovascular invasion. Perineural invasion was present in 55(23.2%) patients with a stage distribution pattern as 69(29.1%) were in stage I and 168(70.9%) were in stage II. Pathological lymph node status

**Table 2** Association between WPOI with various clinicopathological factors

| Variables | No. of Patients | WPOI | | *p*-value |
|---|---|---|---|---|
| | | Group A (1,2,3) | Group B (4,5) | |
| Sex | | | | 0.90 |
| Female | 25 | 12 (10.8%) | 13 (10.3%) | |
| Male | 212 | 99 (89.2%) | 113 (89.7%) | |
| Age | | | | 0.813 |
| 25–40 years | 62 | 27 (24.3%) | 35 (27.8%) | |
| 41–60 years | 132 | 64 (57.7%) | 68 (54%) | |
| 61 + years | 43 | 20 (18%) | 23 (18.3%) | |
| Clinical T stage | | | | **0.001** |
| cT1 | 69 | 44 (39.6%) | 25 (19.8%) | |
| cT2 | 168 | 67 (60.4%) | 101 (80.2%) | |
| Depth of invasion | | | | **0.001** |
| </= 6 mm | 140 | 77 (69.4%) | 63 (50%) | |
| > 6 mm | 197 | 34 (30.6%) | 63 (50%) | |
| Perineural invasion | | | | **0.003** |
| Absence | 48 | 96 (86.5%) | 86 (68.3%) | |
| Presence | 20 | 15 (13.5%) | 40 (31.8%) | |
| Histologic grade | | | | **0.001** |
| Well differentiated | 29 | 26 (23.4%) | 3 (2.4%) | |
| Moderately differentiated | 164 | 72 (64.9%) | 92 (73%) | |
| Poorly differentiated | 44 | 13 (11.7%) | 31 (24.6%) | |
| Tobacco Consumption | | | | 0.941 |
| No | 55 | 26 (23.4%) | 29 (23%) | |
| Yes | 182 | 85 (76.6%) | 97 (77%) | |

on final histopathology report 171(72.1%) were node negative while 66(27.9%) patients had pathological lymph node involvement. Tumor differentiation pattern was 164(69.2%) were moderately differentiated, 44(18.6%) were poorly differentiated and 29(12.2%) were well differentiated.

### Tumor Budding

Low intensity TB was seen in 169 cases (71.3%) followed by high intensity TB in 68 cases (28.7%). TB was significantly associated with age ($p = 0.022$), DOI ($p = 0.003$), histological grade ($p = 0.006$) and worst pattern of invasion ($p < 0.001$), while no significant association was noted with sex, clinical stage, perineural invasion, lymphovascular invasion and tobacco addiction. (Table 1)

#### Worst Pattern of Invasion

WPOI was graded as type 1 to 5. There was significant association between WPOI and clinical tumor stage ($p = 0.001$), DOI ($p = 0.001$), perineural invasion($p = 0.003$), histological grade($p = 0.001$) and tumor budding ($p < 0.001$) while no association between age, gender and tobacco consumption was noted. (Table 2)

### WPOI, Tumor Budding and Regional Lymph Node Metastasis Inter-Relation

In patients with lymph node metastasis, 75.8% had high WPOI compared to 24.2% who had low WPOI. 55.6% of those with low WPOI did not have metastasis compared to 44.4% who had high WPOI, this difference was found to be statistically significant. In patients with lymph node metastasis, 74.2% had high TB compared to 25.8% who had.

low TB, 88.9% of those with low TB did not have metastasis compared to 11.1% who had high TB and this difference was found to be statistically significant. Poorly.

differentiated tumor was seen in 28.8% of those with metastasis and 14.6% of those without metastasis. Moderately differentiated tumor was seen in 66.7% of those with metastasis and 70.2% of those without metastasis. Well differentiated tumor was seen in 4.5% of those with metastasis and 15.2% of those without metastasis. This difference was found to be statistically significant. Almost 57.6% of those with lymph node metastasis had a depth of more 0.6 cm compared to 42.4% who had a depth of less than and equal to 0.6 cm − 65.5% of those without lymph node metastasis had a depth of less than equal to 0.6 cm and 34.5% had a depth of more than 0.6 cm. This difference was found to be statistically significant. (Table 3)

Logistic regression analysis was done to predict the association between the major independent variables- age, gender, tobacco consumption, clinical staging, depth of invasion, histopathology, WPOI, TB and the dependent variable- lymph node status. Nageelkerke R square was obtained at 0.511, Hosmer and Lemeshow goodness-of-fit test computed a Chi-square of 6.429, value of 0.599 with 8 degree of freedom was.

obtained implying that the model's estimates fit the data at an acceptable level. The overall percent correctly predicted was 84%. Higher age group, WPOI and TB were associated with positive prediction of lymph node metastasis which was also found to be significant. Lymph node metastasis in those above 60 years was found to be 0.11 times more compared to those between 25 and 40 years and 0.149 times more in 41–60 years. Also, lymph node metastasis was 4.5 times more likely to occur in those with high WPOI as compared to those with low WPOI and 30.8 times more likely in those in high TB compared to low TB. (Table 4)

**Table 3** Association between regional lymph node metastasis with various clinicopathological factors

| Variables | No. of Patients | Regional lymph node metastasis | | *p*-value |
|---|---|---|---|---|
| | | Yes | No | |
| **Sex** | | | | 0.152 |
| Female | 25 | 10 (15.2%) | (15 (8.8%) | |
| Male | 212 | 56 (84.8%) | 156 (91.2%) | |
| **Age** | | | | 0.288 |
| 25–40 years | 62 | 15 (22.7%) | 47 (27.5%) | |
| 41–60 years | 132 | 42 (63.6%) | 90 (52.6%) | |
| 61 + years | 43 | 9 (13.6%) | 34 (19.9%) | |
| **Clinical T stage** | | | | 0.097 |
| cT1 | 69 | 14 (21.2%) | 55 (32.2%) | |
| cT2 | 168 | 52 (78.8%) | 116 (67.8%) | |
| **Depth of invasion** | | | | **0.001** |
| </= 6 mm | 140 | 28 (42.4%) | 112 (65.5%) | |
| > 6 mm | 97 | 38 (57.6%) | 59 (34.5%) | |
| **Perineural invasion** | | | | 0.13 |
| Absence | 48 | 47 (71.2%) | 135 (78.9%) | |
| Presence | 20 | 19 (28.8%) | 36 (21.2%) | |
| **Histologic grade** | | | | **0.008** |
| Well differentiated | 29 | 3 (4.5%) | 26 (15.2%) | |
| Moderately differentiated | 164 | 44 (66.7%) | 120 (70.2%) | |
| Poorly differentiated | 44 | 19 (28.8%) | 25 (14.6%) | |
| **Worst pattern of invasion (WPOI)** | | | | **< 0.001** |
| WPOI-1,2,3 | 111 | 16 (24.2%) | 95 (55.6%) | |
| WPOI-4 & 5 | 126 | 50 (75.8%) | 76(44.4%) | |
| **Tumour budding** | | | | **< 0.001** |
| Low intensity (< 5 buds) | 169 | 17 (25.8%) | 152 (88.9%) | |
| High intensity (≥ 5 buds) | 68 | 49 (74.2%) | 19 (11.1%) | |
| **Tobacco Consumption** | | | | 0.563 |
| No | 55 | 17 (25.8%) | 38 (22.2%) | |
| Yes | 182 | 49 (74.2%) | 131 (77.8%) | |

**Table 4** Predictive factors for lymph node metastasis

| Variables | Adjusted OR | 95% CI | *P* Value |
|---|---|---|---|
| Age Groups | | | **0.006** |
| 25–40 years | 0.11 | 0.027–0.448 | **0.002** |
| 41–60 years | 0.149 | 0.043–0.516 | **0.003** |
| 61 years and above * | | | |
| WPOI | | | **0.001** |
| High | 4.5 | 1.853–11.305 | |
| Low * | | | |
| Tumor Budding | | | **< 0.001** |
| High | 30.8 | 12.365–76.731 | |
| Low * | | | |

## Discussion

TNM staging and lymph node involvement remains the gold standard for influencing for predicting, prognostication and stratification of oral squamous cell carcinoma. Heterogeneity in survival within the same tumor stage points to the need for additional predictive and prognostic markers. If it is possible to illuminate conceivable clinicopathological parameter and predictors of nodal metastasis, they could be used to identify patients for the management of the neck nodes.

The purpose of the study was to identify predictive factors for cervical lymph node metastasis in early OSCC. The current prospective observational study showed.

interesting results. First, higher intensities of TB and WPOI type 4 and 5 were correlated with regional metastasis in patients with cT1-T2 N0 M0 OSCC. IHC staining.

for cytokeratin can be used to correctly assess the invasive tumor pattern, and WPOI resulting in therapeutic benefits for patients with early OSCC.

## Demographic Consideration

In our study we found most of the patients in age group between 41 and 60 years which concur with the national registries and other studies conducted in India [10, 11]. The preponderance of male cases is likely due to the prevalence of tobacco addiction in male population (70.5%).

## Tumor Budding

TB is countenance of malignancy viz. mislaying of cellular cohesion and active invasion movement. Tumor buds are markers of epithelial-mesenchymal transition which is a molecular process implicated as a hallmark for invasion and metastasis [12].

In the present study TB was significantly associated with age, DOI, histological grade, and WPOI ($p < 0.05$). The importance of TB in cancer prognosis has been studied widely particularly in colorectal cancer [7, 13], where it is recognized as an additional prognostic marker [14]. In esophageal cancer [3], pancreatic cancer [5], and lung cancer [4], TB has been reported as a promising prognostic marker. A significant correlation between high TB count and the presence of lymph node metastasis is one of the most important findings observed in OSCC and in many other cancers [15]. Such a finding stipulate that TB is an early step enroute to metastasis. Another advantage of the studies of TB in OSCC is that their results are consistent with those from the first study that evaluated budding in OSCC [16].

There were very few studies on the correlation of TB with WPOI. We noted a strong correlation between TB and WPOI ($< 0.001$); in contrast, no correlation was noted in a study by Shimizu et al. [12]. TB was found to be a good predictor of clinically node-negative OSCC cases in some studies, further adding its utility as an important histopathological parameter [17]. Angadi et al. [18] reported high-intensity TB to be a strong independent prognostic factor for the prediction of lymph node metastasis.

Most studies evaluated TB using H&E staining. Interestingly, a recent study on OSCC concluded that evaluation of TB by immunohistochemistry with pan cytokeratin antibodies showed a better reproducibility of results than those with H&E staining [12]. In the present study both H& E and IHC staining were used to identify the TB and WPOI. It becomes easy, reproducible, and uniform way to represent the findings which is an important characteristic when considering a new marker for clinical application.

## Worst Pattern of Invasion

Brandwein-Gensler et al. [19] validated that their histologic risk assessment model was strongly predictive for OS and DFS (HR 9.16, 95% CI 2.65, 31.66, $P = 0.0050$). They showed that aggressive pattern of invasion (WPOI-Type 4 and 5) were significantly associated with poorer OS and positive lymph nodes, in comparison to non-aggressive ones (WPOI Type 1–3) in their cohort. Similar to the study by Rodrigues et al. [20], in present study type 4 WPOI was the most common pattern and WPOI- 4 and 5 taken together showed clinical significance when compared with type 1–3. The POI at the advancing front of the tumor and level of differentiation are some of the individual histological parameters that help to predict regional lymph node metastasis [21]. Differentiated neoplastic cells have the tendency to invade the underlying.

connective tissue with pushing, well-delineated borders, the poorly differentiated cells of the tumor possess significantly infiltrative margins [22]. A significant correlation was found between the frequency of metastases and the type of invasive growth pattern [23].

In the present study, WPOI showed significant correlation with clinical T stage, DOI, PNI, histological grade and TB ($p < 0.05$). Similarly, a significant association was reported by Khwaja et al. [22] Seki et al. [24] and Siriwardena et al. [21] Shimuzu et al. [12] also reported a significant relation between WPOI and lymph node metastasis similar to the present study.

## Interrelationship between Tumor Budding and WPOI

There was a strong correlation ($P < 0.001$) between TB and WPOI indicating the higher intensity of TB in higher grades of WPOI and vice versa, similar to results in Shimuzu et al. [12] study.

## Other Histopathological Parameters

In this study there was no correlation between LVI, PNI and neck node metastasis but other studies have found them to be independent predictors [25, 26]. C. Y. Tsai et al. found PNI to be independent predictor for lymph node metastasis [27] .

DOI is an independent predictor of neck node metastasis and in the present study DOI of more than 6 mm is predictive of neck metastasis and statistically significant. When the depth is less or equal to 6 mm then the p value does not come significant reason being relatively smaller number of cases. Tumor DOI is an independent predictor for nodal status in pT1 T2 OSCC. DOI ≥ 4 mm is an indication for an elective neck dissection in pT1 N0 oral squamous cell carcinoma in the study by L J Melchers et al. [28]. Mehmet Haksever et al. [29], showed that the prevalence of neck lymph node metastasis in patients OSCC increases as the tumor depth increases and as the degree of tumor differentiation decreases from well to poor, as has been shown in previous studies [29] .

On analysis there was no significant association between nodal status and sex, clinical T stage, and habits. C. Y. Tsai et al. [27] and d'Alessandro et al. [26] also found no significant association between- sex, age and morphology with final nodal status in their respective study. Krishnamurthy [30] noticed increasing trend of tongue cancers among non-tobacco users in his study.

Patients with higher grade had a higher chances of cervical lymph node metastasis in the current study. Poorly differentiated tumor was seen in 28.8% of those with metastasis and 14.6% of those without metastasis. Moderately differentiated tumor was seen in 66.7% of those with metastasis and 70.2% of those without metastasis. Well differentiated tumor was seen in 4.5% of those with metastasis and 15.2% of those without metastasis. The difference was found to be statistically significant. This was comparable to the study done by Vishak et al. [31]. Various studies have shown grade to be predictor of lymph node metastasis. Byers et al. found that an increasing tumor grade predicted lymph node metastasis (odd ratio2.40) [32]. Sparano et al. and Lim et al. also showed positive correlation between grade of the tumor and lymph node metastasis [25].

## Conclusion

Our study has shown a strong predictive association between regional lymph node metastasis and tumor budding along with WPOI and higher age group. It could be adapted as a routinely assessed predictive marker and mentioned in histopathology reports with its prognostic implications.

Tumor budding and WPOI evaluation can be done in wide local excision specimen for the determination of aggressiveness of the tumor and adequate treatment planning. These neoteric predictors in early-stage OSCC can give a clue for adverse prognosis and thus can be used for risk adjusted follow-up or to plan adjuvant therapy.

A larger study may be required to detect other predictors of lymph node metastasis and to devise a risk score incorporating tumor budding, worst pattern of invasion, depth of invasion, tumor differentiation and T stage to predict a score which can be used to predict to avoid neck dissection in early stage OSCC.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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