Original Article

Relevance of Tumor Budding and Pattern of Invasion in Oral Squamous Cell Carcinoma

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Abstract

Background: Standard histopathological parameters such as depth of invasion (DOI), lymphovascular invasion (LVI), and perineural invasion (PNI) are known parameters that can correlate with the prognosis and aggressiveness of oral squamous cell carcinomas (OSCCs). Tumor budding (TB) (≤5 tumor cells at infiltrating borders) and pattern of invasion (POI) are emerging histopathological parameters that have shown promising results as reliable risk factors in predicting nodal metastasis in early OSCCs. **Aim:** The aim of the study was to assess TB and POI in OSCCs. **Materials and Methods:** A total of 33 surgical resection specimens of OSCC, including buccal mucosa and tongue with neck dissection, were selected. TB and POI along with standard parameters such as grade, DOI, LVI, PNI, lymph node status, and pathological staging were evaluated. These parameters were analyzed in comparison with lymph node involvement and pathological stage of the tumor using the Chi-square and Fischer's exact test. The SPSS software, v21, was used for statistical analyses. **Results:** Most of OSCC were moderately differentiated tumors (63.64%). TB was present in 23 cases, in which 69.57% of cases showed low TB (<5 buds), while 30.43% of cases had higher TB (>5 buds). The worst POI (Patterns 4 and 5) was seen in 75.76% of cases. TB, POI, grade, PNI, DOI, and stromal pattern were significantly associated with the pathological stage of the tumor. **Conclusion:** TB and POI are important and reliable in histopathological parameters in OSCCs.

Keywords: *Oral squamous cell carcinoma, pattern of invasion, tumor budding*

Introduction

Oral cavity cancers are one of the most common cancers in India as well as worldwide. As per the GLOBOCAN 2020, oral cavity cancers rank second in all newly detected cancers, while they are the third-most common cause for cancer-related deaths in India.[1] Oral squamous cell carcinoma (OSCC) is the most common in the oral cavity. In developed countries, tobacco and alcohol consumption are the main risk factors, while in the Indian population, the use of betel areca nuts and snuffing are major risk factors. Incidence in the younger population is also increasing. The prognosis of OSCC is poor, especially with recurrence. Hence, to identify the risk factors is important in predicting the outcome of the OSCC.[2]

OSCCs are one of the important public health problems in India associated with morbidity and mortality. Two important prognostic indicators of OSCC are the stage of the tumor, defined by tumor, node, and metastasis staging system and lymph node metastasis. T1-2, N0, M0 tumors are considered as early-stage OSCC, which is a heterogeneous group associated with variable treatment outcomes and prognosis; hence, further sub-classifying into high-risk and low-risk groups can aid in better management of the cases. Although the patients are classified similarly, their treatment response and prognosis are variable attributed to tumor heterogeneity.[3]

Various clinical and histopathological parameters are being evaluated as novel potential prognostic markers, including depth of invasion (DOI), perineural invasion (PNI), and lymphovascular invasion (LVI). Studies have shown that tumor budding (TB) and pattern of invasion (POI) are important factors in predicting nodal metastasis, especially in early oral squamous cell carcinoma. Thus, they can be evaluated for the stratification purpose of early OSCC.[3] Hence, the current study was undertaken to evaluate TB and POI in OSCCs.

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Materials and Methods

This was a retrospective cross-sectional descriptive study in which we reevaluated all consecutive cases of complete surgical resection specimens of OSCCs with cervical lymph node dissection whose formalin-fixed paraffin-embedded (FFPE) tissue blocks and/or slides are available in the repository of the department of pathology of a tertiary care hospital over 1 year. Oral cavity tumors, including tongue and buccal mucosa, were included. Cases in which patients received preoperative radiotherapy or chemotherapy, cases without lymph node dissection, or where slides or FFPE blocks were not available for review were excluded.

Relevant demographic details and tumor size were noted based on archive records. Pathological staging was reevaluated as per the American Joint Committee on Cancer (AJCC) 8th edition. All pathologists reviewed the selected cases for histopathological assessment of various selected parameters. All pathologists were blinded for the clinical data. The evaluated histopathological parameters included newer parameters such as TB, POI, lymphocyte host response, and stromal response along with standard parameters such as grade of tumor, DOI, LVI, and PNI. Lymph nodes were evaluated for the presence or absence of metastasis, and lymph node status was classified as per the AJCC classification.

POI, TB, and lymphocyte host response were evaluated at the infiltrating tumor borders. TB was defined as an isolated single cell or group of less than five tumor cells in the stroma at the infiltrating tumor fronds. The number of tumor buds per high-power field (40×) was counted in the area of maximum concentration.[4] POI was evaluated as per the criteria described by previous studies which classified them into five patterns. Pattern 1 was defined as broad, pushing well-delineated tumor borders. Pattern 2 was defined as broad, pushing solid bands or fronds. Pattern 3 was recognized as small groups, nests, or cords of invasive tumors with >15 cells per group. Pattern 4 is represented as small groups or islands of cells <15 cells per group and is widely dissociated. Pattern 5 was defined as tumor satellites of size ≥1 mm away from the primary tumor or the closest tumor satellite with intervening normal tissue. The highest pattern was selected in cases of synchronous multiple patterns of invasions. Out of these, POI 4 and 5 were classified as the worst pattern of invasion (WPOI) or invasive/infiltrative pattern, whereas POI 1–3 as a cohesive or noninfiltrative pattern.[5,6] The DOI was measured as the distance between the lowest part of the adjacent normal mucosa and the lowest part of the tumor. The depth was measured in millimeters using a slide caliper and was graded as D1 (≤5 mm), D2 (6–10 mm), and D3 (>10 mm) as classified by Chatterjee *et al*.[3]

Ethical statement

The study was conducted after approval of Institutional ethical committee Of Pravara Institute of Medical Sciences, deemed university vide approval number PIMS/IEC-DR/2022/375 dated on 15.12.2022.

Statistics

All the parameters were analyzed in comparison with lymph node involvement and pathological staging of the tumor as well as with each other using appropriate statistical tests, including the Chi-square and Fischer's exact tests. All statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS, released 2010, version 21.0. Armonk, NY, USA: IBM Corp) software, v21. $P < 0.05$ was considered significant. There were no follow-up data available.

Results

A total of 33 cases were included in the study. Of these, 27 were male, while six were female with an M: F ratio of 4.5:1. The mean age of all cases was 51.79 years with a range from 26 to 72 years. Most of the cases (75.76%) were in the age group of 40–70 years. Out of 33 cases, there were 3 (9.09%) T1, 9 (27.27%) T2, 18 (54.55%) T3, and 3 (9.09%) T4a stage tumors. Moderately differentiated tumors predominated (63.64%), while the rest were well differentiated (36.36%). Lymph node metastasis was identified in 19 (57.58%) cases. Staging of lymph nodes was done as 14 cases-N0, 7 cases-N1, 1 case-N2a, and 11 cases-N2b. Our study included five cases of early-stage (T1/T2, N0, M0) OSCC.

Representative images of TBs, different patterns of invasion, lymphocyte host response, stromal response, LVI, and PNI are shown in Figures 1-3.

The distribution of different parameters as per the pathological stage and lymph node involvement status are shown in Tables 1 and 2, respectively.

Discussion

Histopathology is considered the gold standard for diagnosis of most of the malignancies. The use of various histopathological parameters can provide information about aggressiveness, long-term outcome, and prognosis of malignancies to a limited extent. The use of molecular advances, cytogenetic studies, or immunophenotyping is significantly used for prognostication in a variety of malignancies. However, in countries like India, basic histopathology still can play a vital role in predicting the aggressiveness and prognosis of tumors. Traditional histopathological parameters such as grade/differentiation of tumor, DOI, LVI, PNI, and lymph node status are commonly used to some extent for prognostication. In recent years, the role of the tumor microenvironment and invasive tumor front has grabbed the attention of researchers in predicting the behavior of tumors.

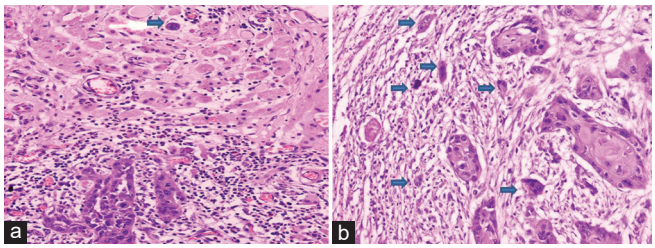

Figure 1: (a and b) Low and high density of tumor buds highlighted by arrows, respectively (×40), H and E

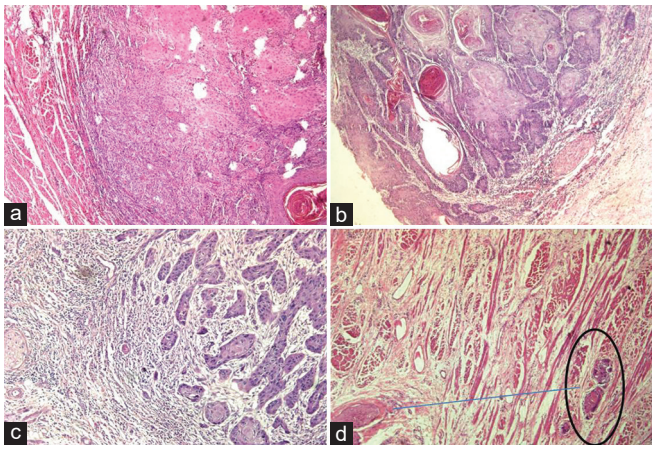

Figure 2: (a) Pattern of invasion 2 (×4), (b) pattern of invasion 3 (×4), (c) pattern of invasion 4 (×4), and (d) pattern of invasion 5, satellite tumor 1 mm away from the main tumor (circle) (×4), H and E

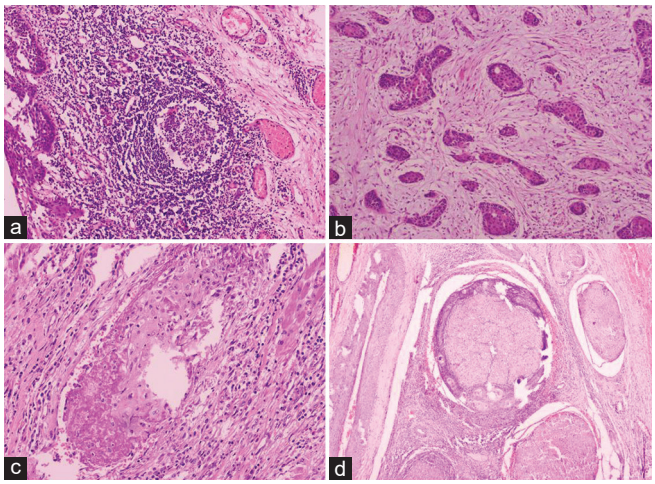

Figure 3: (a) Heavy lymphocyte host response (×10), (b) desmoplastic stroma (×10) (c) lymphovascular invasion (×40) and (d) perineural invasion (×4), H and E

The invasive tumor front is the region located in the tumor border near the stroma which determines the invasive potential. These areas reflect the interaction between the tumor and reactive stromal microenvironment. Parameters such as DOI, POI, and TB are commonly evaluated at these invasive fronts.[7] TB has emerged as a new proposed prognostic marker as well as a marker of disease progression in various solid malignancies such as colon, pancreas, and head-and-neck cancers.[8] TB was first described by

Table 1: The distribution of different parameters as per the pathological stage (pT)

Parameter	T1	T2	T3	T4	*P*
Gender					
Male	3	7	14	3	0.65
Female	0	2	4	0	
Grading					
Well differentiated	2	3	4	3	0.04
Moderately	1	6	14	0	
Poorly differentiated	0	0	0	0	
Pattern of invasion					
Infiltrative (type 4, 5)	0	7	16	2	0.01
Noninfiltrative (type 1, 2, 3)	3	2	2	1	
TB					
Absent	3	3	2	2	0.04
Present	0	6	16	1	
Semiquantitative scoring of TB					
Low TB (<5)	0	5	10	1	0.73
High TB (≥5)	0	1	6	0	
Lymphocyte host response					
Mild	0	1	3	1	0.80
Moderate	2	4	8	2	
Heavy	1	4	7	0	
Stromal pattern					
Loose	2	2	2	2	0.01
Desmoplastic	1	7	16	1	
Hyaline					
LVI					
Absent	3	9	17	2	0.19
Present	0	0	1	1	
PNI					
Absent	3	7	11	0	0.05
Present	0	2	7	3	
Depth group (mm)					
D1 (<5)	2	1	0	0	0.0001
D2 (5–10)	0	8	6	1	
D3 (>10)	1	0	12	2	

PNI: Perineural invasion; LVI: Lymphovascular invasion; TB: Tumor budding

Morodomi *et al*.[9] in 1989 as dedifferentiated cancer cells. Later in 2002, Ueno *et al*.[10] defined TB as isolated single cancer cells or nest of <5 cells at the invasive borders. A TB is a group of isolated cells comprising less than five cells located in the tumor microenvironment, separate from the primary tumor lesion, and located at the infiltrating tumor borders.[4] It has been shown that TB is associated with the epithelial–mesenchymal transition, tumor invasion, and early step in tumor metastasis.[8] Epithelial–mesenchymal transition is suggested by morphological features of TB such as dedifferentiation, cell dyscohesiveness, downregulation of E-cadherin expression, and upregulation of vimentin expression.[2,11] Meta-analyses conducted by Almangush *et al*.,[12] Zhu *et al*.,[13] and Karjol *et al*.[14] have shown that TB is significantly associated with lymph node metastasis, disease-free survival, and overall survival. Higher TB was

Table 2: The distribution of different parameters as per the lymph node involvement status

Parameters	Node free N0 (*n*=14 cases)	Node involved (N1, N2) (*n*=19 cases)	*P*
Gender			
Male	11	16	1.0
Female	3	3	
Grading			
Well differentiated	6	6	0.71
Moderately	8	13	
Poorly differentiated	0	0	
Pattern of invasion			
Infiltrative (4, 5)	9	15	0.44
Noninfiltrative (1, 2, 3)	5	4	
TB			
Absent	4	6	1.0
Present	10	13	
Semi-quantitative scoring of TB			
Low TB (<5)	4	2	0.34
High TB (≥5)	6	11	
Lymphocyte host response			
Mild	1	4	0.58
Moderate	7	9	
Heavy	6	6	
Stromal pattern			
Loose	3	5	1.0
Desmoplastic	11	14	
Hyaline	0	0	
LVI			
Absent	13	18	1.0
Present	1	1	
PNI			
Absent	11	10	0.16
Present	3	9	
Depth group (mm)			
D1 (<5)	1	3	0.64
D2 (5–10)	7	7	
D3 (>10)	6	9	
T-staging			
T1	2	1	0.76
T2	3	6	
T3	8	10	
T4a	1	2	

TB: Tumor budding; PNI: Perineural invasion; LVI: Lymphovascular invasion

associated with lymph node metastasis and poor survival in all these meta-analyses.

Commonly, TB is identified using hematoxylin and eosin stain manually as well as using digital pathology; however, the use of pancytokeratin (AE1/AE3) immunohistochemistry is also used to evaluate TB. Most of the studies have used a ×20 magnification for identification and scoring the highest number of tumor buds. However, some of the studies have used ×40 magnification as ×20 magnification is not universally available for microscopes.[12-14] We used ×40 magnification for identification and scoring TB. TB in OSCC can also be evaluated using digital pathology which can be easy to form standardized scoring system.[15] Most of the studies have stratified TB into low and high TB with a cutoff of <5 and >5 buds, while few Indian studies conducted by Chatterjee *et al*.[3] and Parekh *et al*.[16] used a cutoff of <3 and >3 buds. Xie *et al*.[17] classified TB according to the International Tumor Budding Consensus Conference recommendations using a three-tier system of low, intermediate, and high TB groups and compared with known parameters. Recently, studies such as Almangush *et al*.[18] and Seki *et al*.[19] have highlighted the importance of assessment of TB in preoperative biopsies of OSCC. Seki *et al*.[19] found that TB evaluated using biopsy specimens was a good predictive factor for lymph node metastasis in SCC of the tongue and floor of the mouth. However, few studies have not shown any association between preoperative biopsies.[20]

POI scoring is also being analyzed by various studies for its prognostic role in OSCC positively. The worst POI was proposed by Brandwein *et al*. in 2005. Recent AJCC staging system (8th edition) also includes WPOI-5 assessed at the advancing tumor edge as an important prognosticator in oral cancer.[5,6,21]

Stromal desmoplasia is a reaction of host cells to tumor cell stimuli resulting in the deposition of extracellular matrix and proliferation of fibroblasts. Vucicevic Boras *et al*., in their review, suggested that factors affecting stromal activation such as metalloproteinases, cytokines, growth factors, hypoxia factor, and extracellular adhesion proteins in the stroma can be helpful in profiling and successful management of OSCC.[22,23]

Many studies have evaluated TB as well as POI along with other common histopathological parameters in cases of OSCCs. These studies have shown a significant association between TB and WPOI with lymph node involvement, worse disease-specific survival, and high rates of recurrence.[5,11,16,17,20,21,24-28] However, Chaturvedi *et al*. found a significant association with locoregional recurrence rather than overall survival.[28] Furthermore, Chatterjee *et al*. found a significant association with nodal metastasis rather than with survival.[3] The intensity of TB is also an important factor as studies have shown that higher intensity of tumor buds is associated with poor survival.[11,21,29] Studies have also evaluated these parameters in early- or node-negative OSCC showing significant association with lymph node metastasis or recurrence.[3,7,29] Almangush *et al*.[7] found that DOI, TB, and WPOI can independently predict the prognosis of early OSCC cases which can be used to stratify them into low- or high-risk cases.

We found a significant association between tumor staging (pT) with TB (P = 0.04), POI (P = 0.01), stromal response (P = 0.01), grade of the tumor (P = 0.04), and

DOI (P = 0.0001). However, our study did not highlight the significance of TB and POI in comparison to lymph node involvement compared to most of the literature. The lack of significant results in comparing TB and POI with lymph node metastasis in this study may be attributable to smaller size. We also found a significant association between the presence of TB with POI ($P < 0.00001$) and the grade of the tumor ($P < 0.003216$) in similar lines as studied by Chaitra *et al*.[25] and Xie *et al*.[17] We also found that POI was also significantly associated with the grade of the tumor (P = 0.009). We could not find any significant association between the presence of TB or high TB with early stage (T1/T2, N0, M0) or late stages (node positive or T3/T4) of OSCC (P = 0.6269 and P = 0.5257, respectively).

Limitations of the study

The major limitation of the study was that no follow-up or survival data was available to prognosticate to stratify cases. The sample size was limited. The majority of the cases were of higher stage with a limited number of early-stage OSCCs. No immunohistochemistry was performed, which can be helpful in better and easy identification of tumor buds. Identification of buds can be cumbersome and time-consuming.

However, we found a significant association between tumor stage (pT) with TB, POI, stromal response, grade of tumor, and DOI.

Conclusion

To conclude, TB and POI are simple, reliable, low-cost, and reproducible independent histopathological prognostic parameters in OSCC, which can be included as standard parameters in reporting guidelines in both resection specimens and early biopsy specimens. The usefulness of these newer histopathological parameters should be further better investigated for the prognostication of early OSCCs.

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Conflicts of interest

There are no conflicts of interest.

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