RESEARCH PAPER

Association of Tumor Budding with Histopathological Grade in Oral Squamous Cell Carcinoma: A Cross-Sectional Histopathological Study

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Received: 25th June 2026; **Revised:** 28th July 2026; **Accepted:** 10th October 2026; **Available Online:** 15th October 2026

ABSTRACT

Background:
Tumor budding is an emerging histopathological marker associated with aggressive tumor behaviour in oral squamous cell carcinoma (OSCC). Its relationship with conventional histopathological grading may provide additional information about tumor invasiveness.

Aim:
To evaluate the association between tumor budding and histopathological grade in oral squamous cell carcinoma.

Materials and Methods:

This cross-sectional histopathological study was designed to include 120 histopathologically confirmed cases of OSCC. Hematoxylin and eosin-stained sections were assessed for tumor differentiation and budding at the invasive front. Tumor budding was classified as low (0–4 buds), intermediate (5–9 buds) or high (≥10 buds). Associations were evaluated using appropriate statistical tests, with $p<0.05$ considered significant.

Results:
In the illustrative dataset, tumor budding was identified in 78.3% of cases, with high-grade budding observed in 26.7%. High-grade budding increased from 4.8% in well-differentiated tumors to 23.3% in moderately differentiated tumors and 88.9% in poorly differentiated tumors ($p<0.001$). High-grade budding was also associated with greater frequencies of lymphovascular invasion (43.8%), perineural invasion (37.5%) and non-cohesive invasion (75.0%).

Conclusion:
The illustrative findings suggest that increased tumor budding is associated with poorer histopathological differentiation and aggressive invasive characteristics in OSCC. Tumor budding may be a useful supplementary parameter in histopathological assessment, although confirmation using actual clinical data is required.

Keywords: Oral squamous cell carcinoma, tumor budding, histopathological grading, tumor differentiation, invasive front

How to cite this article: *Dushyantsinh Vala1 Dr. Amit Deshwal2 Dr. Ankita Jain3 Dr. Pratibha Kavle4 Dr. Rajat Panigrahi5* Dr. Ullas Saxena6 Dr. Kapil Paiwal7. Association of Tumor Budding with Histopathological Grade in Oral Squamous Cell Carcinoma: A Cross-Sectional Histopathological Study. Int J Drug Deliv Technol. 2026;16(89s):2182-2187. DOI: 10.25258/ijddt.16.89s.207.*

Source of support: Nil.

Conflict of interest: *None*

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is one of the most common malignancies of the oral cavity and remains a major public health concern, particularly in regions where tobacco consumption and betel quid chewing are prevalent. Histopathological grading is routinely used to assess tumor differentiation and provides useful information about tumor behaviour. However, conventional grading does not always reflect the biological aggressiveness of OSCC. Tumor budding has emerged as a potential histopathological marker that may improve the assessment of invasive characteristics. Keerthika et al. [1] examined intratumoral budding in preoperative biopsies and its prognostic relevance compared with conventional World Health Organization (WHO) grading.

Tumor budding is characterized by the presence of isolated tumor cells or small clusters of up to four tumor cells at the invasive front. It is considered a morphological feature associated with tumor cell dissociation and invasive behaviour. Gopakumar et al. [2] evaluated different tumor budding scoring systems and the role of immunohistochemistry in identifying tumor buds. Velusamy et al. [3] also investigated the prognostic significance of tumor budding in OSCC, supporting its potential usefulness in routine histopathological assessment.

The relationship between tumor budding and established clinicopathological parameters has received increasing attention. Narayanan et al. [4] investigated tumor budding as a prognostic histopathological marker in OSCC, while Thoke et al. [5] examined its association with clinicopathological characteristics using modified International Tumor Budding Consensus Conference criteria. These studies suggest that tumor budding may provide information beyond conventional tumor differentiation.

Tumor budding has also been studied in relation to the worst pattern of invasion and other indicators of aggressive tumor behaviour. Singh et al. [6] assessed the association of tumor budding and invasive patterns with clinicopathological factors in OSCC. Similarly, Hakim et al. [7] evaluated the influence of tumor budding and single-cell invasion on survival in patients with locally advanced OSCC.

Although tumor budding is increasingly recognized as a potentially useful histopathological parameter, its relationship with conventional histopathological grade requires further evaluation. **The present study was therefore undertaken to assess the association between tumor budding and histopathological grade in oral squamous cell carcinoma.**

MATERIALS AND METHODS

Study Design and Setting

The present study is designed as a hospital-based, cross-sectional histopathological investigation in the Department of Oral and Maxillofacial Pathology at a tertiary care dental institution. The study will be conducted after obtaining approval from the Institutional Ethics Committee. Patient confidentiality will be maintained throughout the investigation.

Sample Size

A total of **120 histopathologically confirmed cases of oral squamous cell carcinoma** will be included. The proposed sample size was estimated using a previously reported tumor budding prevalence of 74.4%, with a 95% confidence level and 8% absolute precision. Eligible cases will be selected according to the predefined inclusion and exclusion criteria.

Inclusion Criteria

1. Histopathologically confirmed cases of primary oral squamous cell carcinoma.
2. Patients aged 18 years and above.
3. Biopsy or surgical specimens containing sufficient viable tumor tissue for histopathological assessment.

4. Cases with adequate clinical and histopathological records.

Exclusion Criteria

1. Patients who received chemotherapy or radiotherapy before tissue sampling.
2. Recurrent oral squamous cell carcinoma.
3. Specimens showing extensive necrosis or inadequate tissue preservation.
4. Cases with insufficient tumor tissue or an unidentifiable invasive front.

Histopathological Evaluation

Formalin-fixed, paraffin-embedded tissue sections will be stained with hematoxylin and eosin (H&E) and examined under a light microscope. Tumors will be classified as well-differentiated, moderately differentiated or poorly differentiated OSCC based on conventional histopathological criteria, including keratinization, cellular pleomorphism, nuclear atypia and mitotic activity.

Assessment of Tumor Budding

Tumor budding will be assessed at the invasive tumor front. A tumor bud will be defined as an isolated malignant cell or a cluster containing up to four tumor cells.

The invasive front will initially be examined at low magnification to identify areas showing the greatest budding activity. Tumor buds will then be counted in a selected hotspot using a ×20 objective lens.

For standardized assessment, counts will be normalized to a field area of 0.785 mm². Tumor budding will be classified into three categories:

- **Low budding (Bd1):** 0–4 tumor buds.
- **Intermediate budding (Bd2):** 5–9 tumor buds.
- **High budding (Bd3):** ≥10 tumor buds.

These categories are adapted from the International Tumor Budding Consensus Conference scoring system. Their application to OSCC will be specified as an adapted method rather than a universally established oral cancer grading standard.

Two experienced oral pathologists will independently evaluate the histopathological sections. Disagreements will be resolved through joint microscopic review.

Study Variables

The primary variables will be histopathological grade and tumor budding category. Patient age, sex, tumor location and other available clinicopathological characteristics will also be recorded.

Statistical Analysis

Data will be analysed using SPSS software, version 25.0. Categorical variables will be expressed as frequencies and percentages. The association between tumor budding category and histopathological grade will be evaluated using the chi-square test or Fisher–Freeman–Halton exact test, as appropriate.

The relationship between the ordered categories of tumor budding and histopathological grade will also be assessed using Spearman's rank correlation coefficient. Interobserver agreement will be evaluated using weighted Cohen's kappa statistic.

A **p-value <0.05** will be considered statistically significant.

RESULTS

A total of 120 histopathologically confirmed cases of oral squamous cell carcinoma (OSCC) were included in the illustrative analysis. The mean age was 52.6 ± 11.4 years, with the highest proportion of patients belonging to the 41–60-year age group (55.0%). Males constituted 70.0% of the study population. The buccal mucosa was the most frequently involved site (40.0%), followed by the tongue (30.0%). Moderately differentiated OSCC was the predominant histopathological grade, accounting for 50.0% of cases.

Table 1. Demographic and Clinicopathological Characteristics of Study Participants (n=120)

Parameter	Category	Frequency (n)	Percentage (%)
Age (years)	≤40	24	20.0
	41–60	66	55.0
	>60	30	25.0
Sex	Male	84	70.0
	Female	36	30.0
Tumor location	Buccal mucosa	48	40.0

Parameter	Category	Frequency (n)	Percentage (%)
	Tongue	36	30.0
	Gingivobuccal sulcus	18	15.0
	Floor of mouth	10	8.3
	Other oral sites	8	6.7
Histopathological grade	Well differentiated	42	35.0
	Moderately differentiated	60	50.0
	Poorly differentiated	18	15.0

Table 2. Distribution of Tumor Budding Among OSCC Cases (n=120)

Tumor Budding Parameter	Category	Frequency (n)	Percentage (%)
Presence of tumor budding	Present	94	78.3
	Absent	26	21.7
Tumor budding grade	Low (Bd1: 0–4 buds)	46	38.3
	Intermediate (Bd2: 5–9 buds)	42	35.0
	High (Bd3: ≥10 buds)	32	26.7
Mean tumor bud count	Mean ± SD	6.8 ± 4.3	—

Tumor budding was identified in 94 cases (78.3%), while 26 cases (21.7%) showed no budding. Low-grade budding was observed in 46 cases (38.3%), intermediate budding in 42 (35.0%), and high-grade budding in 32 (26.7%). The illustrative mean tumor bud count was 6.8 ± 4.3.

Table 3. Association Between Tumor Budding and Histopathological Grade

Histopathological Grade	Low Budding Bd1 n (%)	Intermediate Budding Bd2 n (%)	High Budding Bd3 n (%)	Total	p-value
Well differentiated	28 (66.7)	12 (28.6)	2 (4.8)	42	
Moderately differentiated	16 (26.7)	30 (50.0)	14 (23.3)	60	**<0.001**
Poorly differentiated	2 (11.1)	0 (0.0)	16 (88.9)	18	
Total	**46**	**42**	**32**	**120**	

A clear increase in tumor budding was observed with worsening histopathological differentiation in the illustrative data. Among well-differentiated tumors, 66.7% showed low budding, while only 4.8% demonstrated high budding. In contrast, high-grade budding was present in 88.9% of poorly differentiated tumors. Moderately differentiated tumors showed a more variable distribution, with intermediate budding being the most frequent category (50.0%).

The illustrative association between tumor budding and histopathological grade was statistically

significant ($p<0.001$), suggesting that increased budding activity may be associated with poorer tumor differentiation.

Table 4. Relationship Between Tumor Budding and Selected Histopathological Parameters

Histopathological Parameter	Low Budding (n=46)	Intermediate Budding (n=42)	High Budding (n=32)	p-value
Lymphovascular invasion				
Present	4 (8.7%)	9 (21.4%)	14 (43.8%)	<0.001
Absent	42 (91.3%)	33 (78.6%)	18 (56.3%)	
Perineural invasion				
Present	3 (6.5%)	7 (16.7%)	12 (37.5%)	0.002
Absent	43 (93.5%)	35 (83.3%)	20 (62.5%)	
Pattern of invasion				
Cohesive	38 (82.6%)	24 (57.1%)	8 (25.0%)	<0.001
Non-cohesive	8 (17.4%)	18 (42.9%)	24 (75.0%)	

High-grade tumor budding was associated with a greater frequency of adverse histopathological characteristics in the illustrative dataset. Lymphovascular invasion was observed in 43.8% of high-budding tumors compared with 8.7% of low-budding tumors. Similarly, perineural invasion was present in 37.5% and 6.5% of cases, respectively. A non-cohesive pattern of invasion was also more common among tumors exhibiting high budding activity (75.0%).

These patterns suggest that increased tumor budding may accompany more aggressive histopathological features in OSCC.

DISCUSSION

Tumor budding has gained attention as a histopathological indicator of invasive behaviour in oral squamous cell carcinoma (OSCC). In the present illustrative analysis, tumor budding was identified in 78.3% of cases, with high-grade budding observed in 26.7%. A significant association was observed between increasing tumor budding and poorer histopathological differentiation ($p<0.001$). High-grade budding was particularly frequent in poorly differentiated tumors (88.9%), suggesting that tumor budding may reflect aggressive histological characteristics. Vieira E Silva et al. [8], in their meta-analysis, identified tumor budding as an adverse prognostic factor in head and neck squamous cell carcinoma, supporting its potential value in histopathological assessment.

Feitosa et al. [9], in a systematic review and meta-analysis, examined the relationship between tumor budding and prognosis in oral cancer. Their findings indicated that increased tumor budding was associated with unfavourable clinical outcomes. The present illustrative results showed a similar pattern of greater budding activity in tumors with poorer differentiation. However, the cross-sectional design does not permit conclusions regarding survival or disease recurrence.

The relationship between tumor budding and other adverse histopathological features was also examined. High-grade budding was associated with increased frequencies of lymphovascular invasion (43.8%) and perineural invasion (37.5%). Yadav et al. [10] investigated tumor budding in relation to histomorphological prognostic markers and epithelial–mesenchymal transition in OSCC. Their work provides a biological explanation for the association between tumor budding and invasive tumour characteristics, as budding cells may exhibit reduced intercellular adhesion and increased migratory potential.

The illustrative analysis further demonstrated that tumor budding increased with worsening histopathological grade. A previous study examining tumor budding and invasive characteristics in OSCC grading [11] also considered the relevance of invasive-front

morphology in assessing tumor aggressiveness. Conventional histopathological grading primarily reflects cellular differentiation, whereas tumor budding provides additional information about the pattern of invasion. Combining these parameters may therefore improve the morphological assessment of OSCC.

High-grade tumor budding was also associated with a non-cohesive pattern of invasion, which was observed in 75.0% of high-budding tumors compared with 17.4% of low-budding tumors. A study conducted at a regional cancer centre in Central India [12] evaluated tumor budding and the worst pattern of invasion in relation to lymph node metastasis and disease-free survival. Similarly, another investigation [13] examined these histopathological parameters as predictors of lymph node metastasis in early OSCC. These studies suggest that tumor budding and invasive growth patterns may provide complementary information about tumor behaviour.

Although tumor budding has been associated with adverse clinical outcomes, its relationship with individual prognostic parameters remains uncertain. A systematic review and meta-analysis [14] reported that tumor budding was associated with overall survival but did not establish a consistent association with lymph node metastasis. This distinction is relevant because histopathological features associated with aggressive morphology do not necessarily predict every clinical outcome. The present study was limited to histopathological associations and did not evaluate survival or recurrence.

The incorporation of tumor budding into histopathological grading has also been explored. A study evaluating a grading system based on tumor budding and cell nest size [15-17] reported favourable interobserver and intraobserver agreement. Such approaches may help address some limitations of conventional differentiation-based grading, particularly in tumors showing heterogeneous morphology.

The main limitations of the proposed study include its single-centre design, relatively small number of poorly differentiated tumors and absence of long-term clinical follow-up. Tumor budding assessment may also be influenced by tissue sampling, inflammatory infiltrates and variability in identifying the invasive front. Standardized scoring and independent evaluation by experienced pathologists would help improve reproducibility. Larger studies incorporating clinical outcomes are needed to establish the additional prognostic value of tumor budding beyond conventional histopathological grading.

CONCLUSION

The illustrative findings suggest that increased tumor budding is associated with poorer histopathological differentiation and adverse invasive characteristics in OSCC. Tumor budding may serve as a useful supplementary parameter in routine histopathological assessment. Its clinical value should be confirmed through analysis of actual patient data and studies incorporating long-term outcomes.

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