Original Article

Significance of tumour budding and invasive characteristics in grading of oral squamous cell carcinoma

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Abstract

Background: Tumour budding has been recognized as a morphologic marker of tumour invasion. Invasive characteristics such as depth of invasion, mode of invasion and worst pattern of invasion are potentially powerful parameters predicting the regional metastasis.

Aim: This study was done to understand the significance of tumour budding and various characteristics of invasion and their impact on grading of oral squamous cell carcinoma.

Materials and Methods: An immunohistochemical study was performed on tissue sections obtained from 34 paraffin-embedded blocks of clinically and histologically diagnosed cases of oral squamous cell carcinoma. The sections were stained with pan cytokeratin and observed under high power magnification.

Results: Tumour budding and the invasive patterns were found to be significant in OSCC. A proposed grading system based on tumour budding and cell nest was found to have a significant correlation with the WHO grading system.

Conclusion: This study demonstrated the importance of using tumour buds as an additional parameter in the grading system and also assessed the importance of invasive patterns, cellular atypia and stromal contents in OSCC.

Keywords: Depth of invasion, mode of invasion, oral squamous cell carcinoma, tumour budding

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common oral cancer, accounting for more than 90% of the cases that showed varying degrees of histological differentiation with high invasive and metastatic potential.[1] The overall survival rate of OSCC has remained less than 50% for more than a decade, despite advances in diagnosis and therapy.[2] Treatment plan and prognosis of OSCC are primarily based on the clinical staging and histologic grading system. The American Joint Committee on Cancer (AJCC) in cooperation with the TNM Committee of the Union for International

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Cancer Control (UICC) created the TNM staging system, which is used as the international standards for cancer recording, staging, prognosis, treatment planning and in therapeutics.[3] Although the system has been widely accepted, several studies have shown that the stratification of each patient prognosis lacks predictive accuracy to differentiate between high-risk groups and low-risk groups based on lymph node metastasis, local recurrence and patient survival.[4,5] A few studies have suggested that the major disadvantage in the use of TNM staging system is in the early-stage tongue squamous carcinoma, because of its high tendency for occult loco-regional metastasis.[6]

The WHO grading system is the most commonly used histologic grading system. According to the degree of differentiation, the tumours have been graded histologically as well, moderately and poorly differentiated OSCC.[7] Even though the WHO grading system has been used commonly, several studies have shown an inadequate correlation with the outcome and treatment response in individual patients. Small incisional biopsy tissues taken from a tumour that has diverse histological heterogeneity, sampling inadequacy, tumour structural characteristics dependency instead of functional ones, limited tumour cell assessment in host tissues and the surrounding stroma were the suggested reasons for the inaccuracy in the grading system.[8] To improve on this, in the recently updated eighth edition of the AJCC staging manual, the depth of invasion (DOI) was added to the T category, and the worst pattern of invasion (WPOI) was also added as an additional factor.

Tumour budding was initially designated as tumour sprouting to the stroma and was first reported in 1954 by Imai.[9] Recent studies have shown the presence of tumour budding as a promising prognosticator in OSCC. Elseragy *et al.* examined a series of early tongue SCC and reported that the addition of tumour budding to the WHO differentiation criteria has a better prognostic value than the conventional WHO system.[10] Tumour budding has been reported to be an independent prognostic factor for several cancers such as colorectal, pancreas, oesophageal, pulmonary and gastric cancer.[11-16]

This study is aimed to assess the various invasion patterns and tumour budding in OSCC and to compare a newer grading system that uses tumour budding, with that of the WHO grading system. In addition to these, cellular atypia (nuclear diameter, mitotic count and degree of keratinization) and stromal content were also assessed which reflects the behavioural pattern of tumour cells.

MATERIALS AND METHODS

Paraffin-embedded archival blocks of 34 cases of OSCC patients who underwent incisional biopsies were taken from the Department of Oral and Maxillofacial Pathology and were graded according to WHO criteria. Twelve cases of well-differentiated OSCC, 12 cases of moderately differentiated OSCC and 10 cases of poorly differentiated OSCC were taken for the study. All specimens were obtained with prior informed consent, and the study protocol was reviewed and approved by Institutional Ethics Committee of PMS College of Dental Science and Research, Thiruvananthapuram (IEC No. PMS/IEC/2022/REGULAR/APR/45).

Immunohistochemistry (IHC) procedure was used for the detection of tumour cells, wherein PAN cytokeratin (AE1/AE3) was used as the primary antibody and Poly Excel Poly HRP (pre-diluted, PEH002) was used as secondary antibody. Sections of 3μm thickness were mounted on APES-coated slides. These sections were deparaffinized in xylene and rehydrated through graded alcohol solutions, followed by wash with distilled water. For antigen retrieval, the slides were immersed in Tris/EDTA buffer and then subjected to steam pressure for 10-15 minutes. The slides were cooled and incubated with hydrogen peroxide for 10 minutes to block the endogenous peroxidase activity. The slides were subsequently incubated with the primary antibody (CK AE1/AE3-PathnSitu) for 45 minutes at room temperature. This was followed by incubation with secondary antibody for 20 minutes. The reaction was visualized with diaminobenzidine (DAB). All slides were counterstained with Mayer's haematoxylin, dehydrated and mounted.

ASSESSMENT OF TUMOUR BUDDING AND INVASIVE PATTERNS

Immunohistochemically stained sections were evaluated using light microscopy. Tumour budding was assessed in areas showing maximal budding activity (BA) and was scored in both one high power field (HPF) and in 10 HPFs. In 1 HPF, low BA was defined as 1-4 budding nests and high BA as five budding nests. In 10 HPFs, low BA was defined as 1-14 nests and high BA if ≥15 budding nests were detected. Cell nests were defined as clustered tumour cells, surrounded by stroma and were classified based on the size of the smallest invasive cell nest. Clusters of >15 tumour cells were classified as large cell nests, 5-15 tumour cells as intermediate cell nests, 2-4 tumour cells as small cell nests, and single-cell invasion were stated for single tumour cell. Cell nest size (CNS) was assessed both at the invasive margin and within the tumour core region.[17]

Mode and pattern of invasion were graded based on criteria by Jakobsson *et al*[18]. DOI is measured by first finding the "horizon" of the basement membrane of the adjacent squamous mucosa. A perpendicular "plumb line" is established from this horizon to the deepest point of tumour invasion, which represents DOI. DOI is recorded in millimetres.[19-21]

Nuclear diameter of tumour cells was assessed within the tumour core region, which harbours the largest nuclei. The scoring was given by comparing the nuclei of small tumour-associated lymphocytes as the reference. If the largest nuclei of tumour cells measures ≤3 lymphocytes, then the score given was small, nuclei roughly matching the diameter of four lymphocytes were scored as intermediate, and nuclei with a diameter of >4 lymphocytes were classified as large. Tumour area with the highest mitotic activity was taken, and its frequency was counted in 10 HPFs (40x) and scored as <10, 10-20, 20-30, 30-40 and >40 in each field. The degree of keratinization was scored as weak (focal or single-cell keratinization), intermediate and strong (keratinization covering >30% of the whole tumour cell area). The amount of tumour-stromal content was classified as very low (<10% of the whole tumour area), low (>10% to 25%), moderate (>25% to 50%) or high (>50%).[17]

In analogy to Boxberg *et al*., scores for BA (1: no budding/10 HPFs; 2: <15 budding foci/10 HPFs; 3: ≥15 budding foci/10 HPFs) and CNS within the tumour core region (1: >15 cells; 2: 5-15 cells; 3: 2-4 cells; 4: single-cell invasion) were assigned. These two variables were summed up to obtain a grading score ranging from 2 to 7. Based on this analysis of sum scores, tumours with scores 2-3 were graded as well-differentiated (G0), with scores 4-6 as moderately differentiated (G1) and with score 7 as poorly differentiated (G2).[17] Table 1 shows the proposed grading system for OSCC using BA and CNS.

Statistical analysis

Fisher's exact test was used to evaluate the correlations among tumour budding, mode of invasion, the worst pattern of invasion, depth of invasion and the histologic parameters such as cellular atypia and stromal contents. Multiple regression equation is used to find the significant linear relationship between the parameters and also BA and CNS with the proposed tumour grading. Agreement between the WHO and proposed grading system that considers BA and CNS was done with Cohen Kappa statistics. *P* value <0.05 was considered statistically significant.

Table 1: Proposed grading system for oral squamous cell carcinoma (OSCC) using tumour budding[17]

	Score
Tumour budding activity (BA)/10 HPF	
No budding	1
<15 budding foci	2
>15 budding foci	3
Smallest cell nest size (CNS)/10 HPF	
>15 cells	1
5-15 cells	2
2-4 cells	3
Single-cell invasion	4
Tumour Grading	
Well-differentiated (G0)	2-3
Moderately differentiated (G1)	4-6
Poorly differentiated (G2)	7

RESULTS

According to the current WHO grading system, 35.29% (12/34) were well-differentiated OSCC, 35.29% (12/34) were moderately differentiated OSCC and 29.41% (10/34) were poorly differentiated OSCC. A small nuclear diameter of tumour cells was seen in 12% of OSCC (4/34), intermediate nuclear size in 15% (5/34) and large nuclear size in 73% (25/34) of cases. A weak degree of keratinization was noticed in 23.5% (8/34), intermediate keratinization in 23.5% (8/34), and strong keratinization in 53% (18/34) of cases. Very low stromal content was seen in 26% (9/34), low in 6% (2/34), moderate in 15% (5/34) and heavy stroll content in 53% (18/34) of cases. Type 1 worst pattern of invasion was seen in 15% (5/34) of cases, type 2 in 9% (3/34), type 3 in 15% (5/34), type 4 in 32% (11/34) and type 5 in 3% (10/34) of cases. Grade I mode of invasion was seen in 17.64% (6/34), grade 2 in 3% (1/34), grade 3 in 23% (8/34), grade 4C in 18% (7/34) and grade 4D in 35% (12/34) of cases. The depth of invasion was assessed only in three cases due to unavailability of adequate tissue specimens, which has the values between 5 and 10 mm [Figures 1 and 2].

A significant correlation was noted between tumour budding and nuclear diameter, degree of keratinization, stromal content and mitotic count ($P < 0.05$). Tumour budding also showed significant positive correlation with mode of invasion and worst pattern of invasion ($P < 0.05$). Mode of invasion showed high correlation value (0.9416) and exhibited significant linear regression relationship with WPOI [Table 2].

Tumour buds were assessed in both low power magnification (10x) and high power magnification (40x) [Figures 3 and 4]. Budding activity (BA) and cell nest size (CNS) were significantly associated with tumour grading ($P < 0.05$). According to our proposed grading

Table 2: Multiple regression equation showing the significant linear relationship between the parameters

Variable	Correlation	*P*	*F* calculated	*F* significant
Budding activity versus				
DOK	0.53	0.0013	12.34	0.0013
Stromal	0.497	0.0028	10.51	0.0028
WPOI	0.412	0.0155	6.53	0.0155
MOI	0.45	0.0074	8.17	0.007446
Nuclear	0.42	0.0118	7.13	0.01183
DOK versus				
Stromal	0.7128	0.000002244	33.0569	0.000002244
WPOI	0.7186	0.000001701	34.1637	0.000001701
MOI	0.7121	0.000002322	32.9224	0.000002322
Nuclear	0.7190	0.000001664	34.2514	0.000001664
Stromal versus				
WPOI	0.6174	0.000100337	19.7126	0.0001003
MOI	0.6862	0.000007482	28.4693	0.000007482
Nuclear	0.7095	0.000002625	32.4398	0.000002625
WPOI versus				
MOI	0.9416	1.09×10^{-15}	250.4299	1.09×10^{-15}
Nuclear	0.7429	4.9×10^{-6}	39.4156	4.9×10-6
MOI versus				
Nuclear	0.7403	5.6×10^{-6}	38.8117	5.6×10^{-6}

DOK=Degree of keratinization, Stromal=Stromal content, WPOI=Worst pattern of invasion, MOI=Mode of invasion, Nuclear=Nuclear diameter

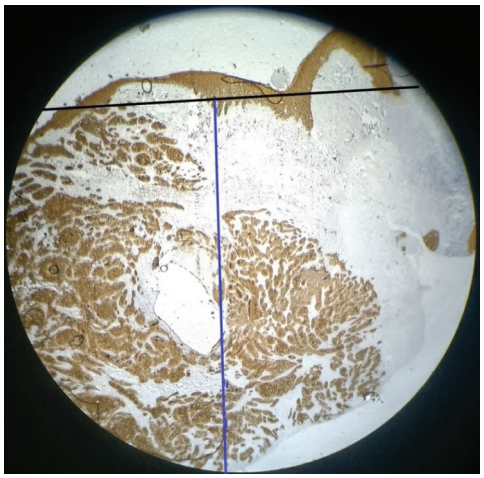

Figure 1: Representation of depth of invasion (7 mm); black line indicates the horizon of the basement membrane and blue line indicates the perpendicular plumb line established from the horizon

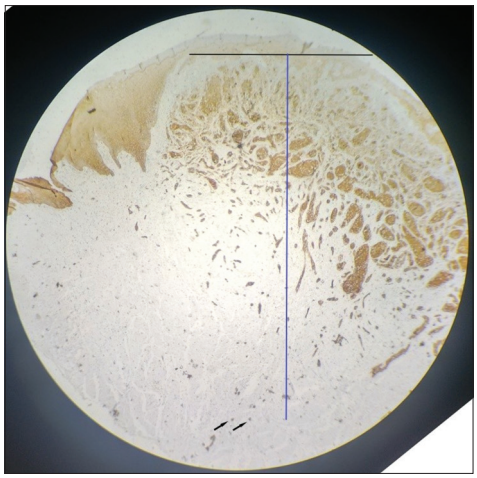

Figure 2: Representation of depth of invasion (6 mm). Black line indicates the horizon of the basement membrane and blue line indicates the perpendicular plumb line established from the horizon. Black arrows indicates tumour buds

system in this study, 32.35% (11/34) of cases were well-differentiated OSCC, 35.29% (12/34) were moderately differentiated OSCC and 32.35% (11/34) were poorly differentiated OSCC. Cohen Kappa statistics revealed good agreement (72%) between WHO and the newly proposed grading system that considers BA and CNS [Figure 5].

In this study, low-grade tumours showed small and intermediate nuclear diameter, while high-grade tumours showed intermediate and large nuclear diameters. Majority of low-grade tumours showed very low and low stromal content. The degree of keratinization was weak in all the well-differentiated OSCC, while the moderately differentiated and poorly differentiated tumours showed both intermediate and strong degree of keratinization. Type 1 and 2 WPOI were seen only in low-grade tumours, while types 3, 4 and 5 were seen in high-grade tumours. Among the 10 cases, which shows type 5 WPOI, six cases showed more tumour buds with single-cell invasion, which indicates the aggressive behaviour of the tumour cells.

DISCUSSION

The first quantitative histopathological grading system for OSCC was initiated by Broders, which is still accepted by the WHO.[22] However, several studies show lack of correlation between Broders' degree of differentiation and prognosis.[23-25] The main reason suggested for the poor correlation was the relative heterogeneity of the tumour cell population with the differences in degree of differentiation. Later, Jakobsson *et al.* (1973) developed a multi-factorial

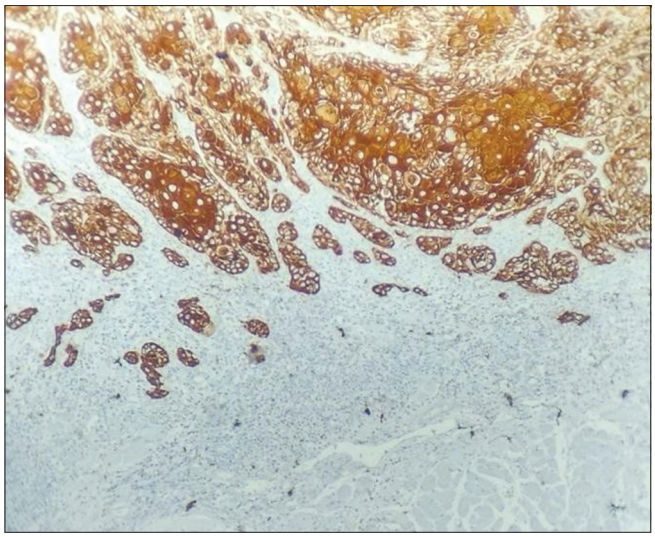

Figure 3: Tumour budding in oral squamous cell carcinoma (OSCC) in 10x magnification

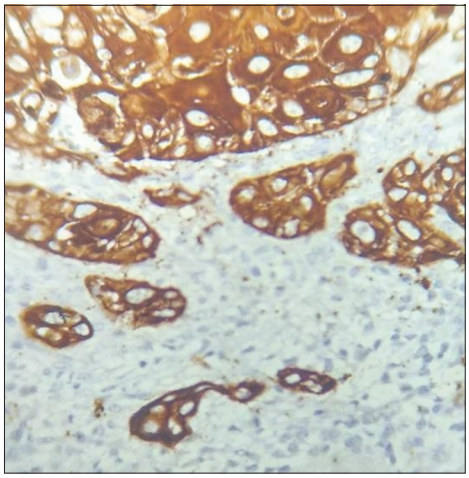

Figure 4: Tumour budding in oral squamous cell carcinoma (OSCC) in 40x magnification

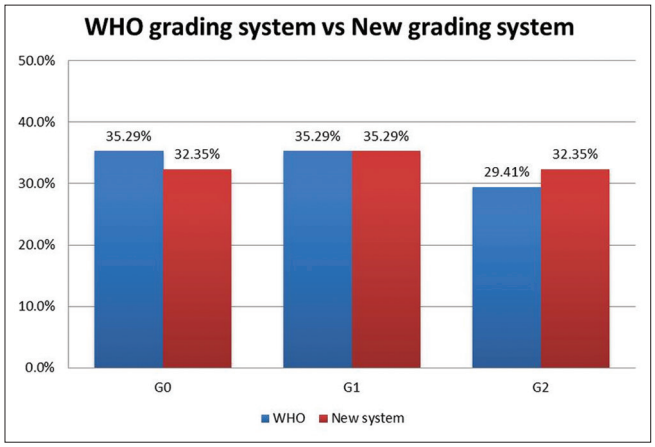

Figure 5: Graph showing the correlation between the WHO grading system and the proposed new grading system

malignancy grading system.[18] Anneroth *et al.* (1984) modified Jakobsson's grading system of SCCs in the tongue and the floor of the mouth.[26] Bryne *et al.* (1989) observed that the cells in the deep invasive margin, termed as 'invasive front' frequently showed a lower degree of differentiation and higher grade of cellular dissociation.[27-29]

Invasive front (IF), which is considered to be particularly important for tumour progression, is the zone of active invasion and important crosstalk between tumour and stroma.[30] Tumour budding is a non-proliferating, non-apoptotic, and highly aggressive subpopulation of tumour cells that display migratory and invasive capacities.[31] Tumour budding is defined as the presence of single carcinoma cell or small clusters of cells (≤5 cells) located at the IF of epithelial tumours. Tumour budding exhibits two characteristic features of malignancy such as: loss of epithelial cell cohesion and active invasion potential. The presence of tumour buds at the invasive front reflects the aggressive behaviour of OSCC.[32] The presence and prognostic significance of tumour budding in OSCC were identified in numerous studies.[33-35] A high tumour bud count at the invasive front has been linked to a poor prognosis and a high risk of metastases in several cancer types, including OSCC.[36-44] A few studies have shown a significant correlation between WPOI and lymph node (LN) metastasis.[45,46] Numerous studies have found correlation between depth of invasion and lymph node metastasis.[47-53]

In this study, we investigated the histopathologic features that were previously incorporated in a novel grading scheme in oral SCC, pulmonary SCC and oesophageal squamous cell carcinoma.[54,55] In analogy to these studies, a simple grading scheme incorporating the two histomorphological characteristics, such as BA and CNS, were used in our study. Although tumour budding and cell nest size are closely related, they influence the malignant potential of a tumour from different angles. Cell nest size within the tumour core captures the tumour's overall capability of cellular discohesion, and BA represents the invasive potential of an OSCC into surrounding tissue within the region of the highest aggressiveness. In our study, tumour budding activity was more with high-grade tumours than in well-differentiated OSCC. Additionally, in a few well-differentiated tumours, single tumour cell invasion was evident, suggesting a more aggressive behavioural nature of the tumour. A proposed grading system based on tumour budding and cell nest has a significant correlation with the WHO grading system.

CONCLUSION

The prognosis and treatment plan of OSCC can be predicted by evaluating the histological parameters like

tumour budding and the invasive characters such as the mode of invasion, worst pattern of invasion and depth of invasion, all of which can be assessed on routine haematoxylin and eosin stains. This study has evaluated the importance of using tumour budding in the grading system along with other parameters. Thus, based on previous studies, and through our study, we suggest the importance of using tumour budding as an important parameter in the tumour grading system. Along with this, we also recommend the minimum requirements of preoperative biopsy specimen, to evaluate the depth of invasion, which can help in the treatment modality of OSCC.

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Conflicts of interest

There are no conflicts of interest.

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