RESEARCH PAPER

A Cross-Sectional Study Of Tumor Budding In Oral Squamous Cell Carcinoma And Its Association With Various Prognostic Variables.

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Abstract:
BACKGROUND: The management and prognosis of Oral Squamous Cell Carcinoma (OSCC) depends on tumor stage and lymph node metastasis status. Many histopathological prognostic parameters like tumor grade, depth of invasion, perineural invasion and lymphovascular invasion have been identified as paramount for planning an appropriate management. Some novel parameters like tumor budding have been identified as aids in predicting the behaviour of OSCC, if included in pathology report.
METHODS: A cross-sectional study was carried out on 40 cases of histologically diagnosed SCC involving the Head and Neck region, received in the pathology laboratory of our institute from January to December 2019. The histopathological findings and prognostic variables were analysed with respect to tumor budding by Pearson Chi-square test.
RESULTS: In this study, low tumour budding score was significantly associated with a lower tumor (pT) stage. However, the other prognostic parameters like tumour grade, depth of invasion, perineural and lymphovascular invasion were not significantly associated with tumor budding in oral SCC.
CONCLUSION: This study attempted to assess the utility of tumour budding as a prognostic factor in relationship to other features. Standardization of the scoring methods for tumour budding will make it as a valuable prognostic parameter in oral SCC.
Keywords: Oral cancer, Tumor buds, Prognostic factor, Stage, Grade, Metastasis.
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INTRODUCTION:

Oral Squamous Cell Carcinoma (OSCC) is the most common malignancy of the oral cavity and constitutes the majority of head and neck squamous cell carcinomas.(1) The incidence is higher in people aged more than 50 years, often associated with chronic exposure to tobacco, alcohol, and other carcinogenic products.(2)

In general, early OSCC is amenable to surgical resection or radiotherapy. Surgical resection is often the definitive treatment in early stage cancer of the oral cavity. However, some patients who were considered curable eventually suffer from loco-regional recurrence after definitive surgery. In order to prevent relapse, several novel interventions are being performed based on new basic research and clinical data.(2)

Several histopathological prognostic indicators of oral cancer have been reported recently, even though these oral cavity cancers show high risk of locoregional recurrence because of the heterogeneity of the disease and the patients.(2) Recently, the utility and significance of tumor budding in histopathological examination is being evaluated for several early stage cancers such as colon, oesophagus and gastric cancers. Tumor budding, defined as the presence of single cancer cells or small clusters of fewer than five cells at the invasive front (IF), indicates the dissociation of invasive cancer cells from the main tumour mass. (1,2)

Studies have shown improved survival rate in OSCC patients treated with adjuvant chemo-radiotherapy along with radical surgery. Identification of factors associated with good outcome could help in assessing the benefits of adjuvant chemotherapy for individual patients. Pathological staging is one such crucial factor which helps to decide on and tailor the adjuvant therapy so as to improve the quality of life of these patients.(3)

The most frequently employed staging system for oral cancer is the Union for International Cancer Control (UICC)/American Joint Committee on Cancer (AJCC) (TNM) staging system. According to the TNM staging system, patients with oral cancer are classified into various stages based on the depth of primary tumor invasion (T stage), regional lymph node metastases (N stage) and distant metastases (M stage).(3)

Many clinical research papers have been published stating the prognostic significance of tumour budding in oral cancers. Most of them suggest that tumour

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budding can be considered to have a better prognostic value than the TNM staging.[4] This study is aimed at assessing the prognostic significance of tumour budding in patients with oral squamous cell carcinoma.

MATERIALS AND METHODS:

This cross-sectional study was carried out in the department of pathology of a tertiary care hospital after obtaining approval from Institutional Ethics Committee. Hematoxylin & Eosin (H&E) stained sections from excisional biopsies of 40 OSCC patients diagnosed and treated in our institution from January to December 2019 were retrieved for this analysis. For all cases, the clinical diagnosis was confirmed based on incisional biopsy report and were treated by surgical excision along with neck dissection. Preoperative biopsies taken from the edge of tumor, with an inadequate representative area, insufficient volume and with artefacts were excluded. Patients with preoperative chemotherapy or radiotherapy and recurrent carcinoma were excluded.

The tumor stage was defined according to the TNM classification of the UICC. In addition, other clinical features such as age, sex, site and size of tumor were also obtained from the patients' file retrieved from the archives of the institution. The paraffin embedded 3-5microns sections stained with Hematoxylin and Eosin (H&E) were studied for histopathological features. The number of isolated tumor cells or small clusters with less than five tumor cells at the invasive tumour front in the area with highest tumor budding intensity was counted at 40x magnification. Tumor buds were evaluated according to scoring criteria of Almangush A et al.[1] To eliminate bias, each section was evaluated by two observers and then jointly by consensus. During the scoring of Tumor Budding (TB) 'B', the whole tumor area was scanned at low magnification (4x) then the highest number of TB was counted at a higher magnification (40x). The cut off point for TB was set at 5 buds (low <5 buds; high ≥5 buds). Depth of invasion (DOI) 'D' was measured from the tumor surface to the deepest point of invasion. The cut off point for depth of invasion was set at 4 mm (low <4 mm; high ≥4 mm). Tumor Budding (TB) 'B' and depth of invasion (DOI) 'D' were taken together as BD score according to BD model by Almangush A et al.[1] In accordance with the BD model, each case was assigned a score between 0 and 2. Cases with score 0 were classified as low risk, those with score 1 as inter-mediate risk and those with score 2 as high risk.

STATISTICAL ANALYSIS:

Statistical analysis was performed using SPSS software, version 23. Descriptive analysis was employed to summarize patients' clinical data such as age, gender, site of lesion, TNM staging and histopathological grading. Categorical data were measured using frequency and percentage. Pearson Chi-Square test was used to test possible relationship between various clinicopathological variables and BD score. Significance level was set at $P < 0.05$.

RESULTS:

The study consisted of 40 cases of OSCC. Patients' age ranged from 30 to 72 years with majority of cases encountered in the age group of 41-60 years (42.5%). There was striking male predominance in the current study with male: female ratio of 3.4:1. The most common site of lesion was buccal mucosa (42.5%) while the least common involved site was retromolar trigone (05%). As far as TNM staging is considered, majority of cases belonged to stage II (32.5%) followed by stage IV (30%). [Table 1]

Histological grading of OSCC revealed majority of well differentiated OSCC (65%) followed by moderately differentiated OSCC (35%) without any poorly differentiated OSCC in this study. Strong lymphocytic host response was noted in 65% of cases and worst pattern of invasion (type 5) was observed in only 10% of OSCCs in the present study. On assessing the tumor budding score in H&E stained sections, 18 cases (45%) had BD score 2, 15 cases (37.5%) had BD score 1 while 7 cases (17.5%) didn't reveal any tumor buds (BD score 0) [Table 1; Figure 1]. Lymphovascular invasion was observed in only 12.5 % cases of OSCC while perineural invasion was observed in only 15% of OSCC cases in the current study [Table 1].

Clinicopathological variables	No of cases (n=40)	Percentage (%)
Age (years)		
• 30-40	15	37.5%
• 41-60	17	42.5%
• >60	8	20%
Gender		
• Males	31	77.5%
• Females	9	22.5%
Site distribution		
• Buccal mucosa	17	42.5%
• Tongue	9	22.5%
• Alveolus	4	10%
• Gingivobuccal sulcus	4	10%
• Floor of mouth	4	10%

• Retromolar trigone	2	05%
Clinical Stage		
• Stage I	9	22.5%
• Stage II	13	32.5%
• Stage III	6	15%
• Stage IV	12	30%
Histological Grade		
• Well differentiated	26	65%
• Moderately differentiated	14	35%
• Poorly differentiated	0	0
Pattern of Invasion		
• Type 1	7	17.5%
• Type 2	5	12.5%
• Type 3	10	25%
• Type 4	14	35%
• Type 5	4	10%
Lymphocytic Host Response		
• Strong	26	65%
• Intermediate	7	17.5%
• Weak	7	17.5%
Tumor budding (BD Score)		
• Score 0 – Low risk	07	17.5%
• Score 1- Intermediate risk	15	37.5%
• Score 2 – High risk	18	45%
Perineural Invasion (PNI)		
• Absent	34	85%
• Present	6	15%
Lymphovascular Invasion (LVI)		
• Absent	35	87.5%
• Present	5	12.5%

Table-1: Clinicopathological characteristics of oral squamous cell carcinoma

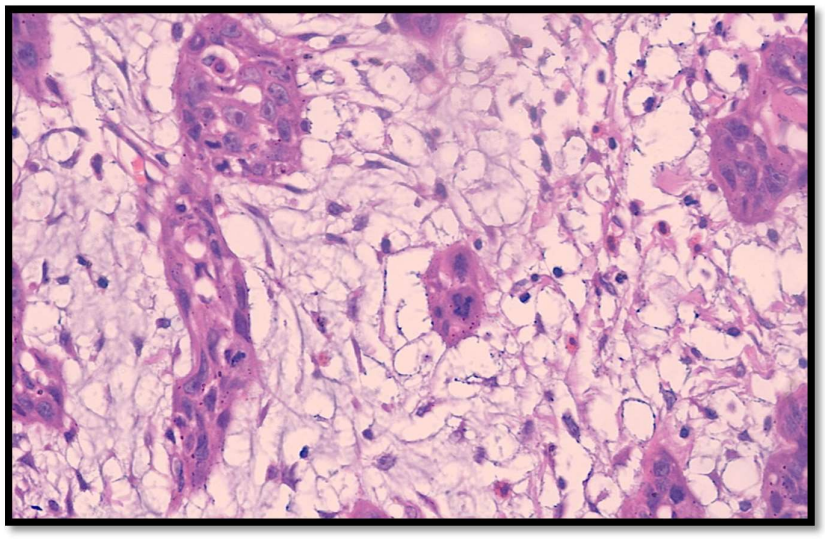

Figure-1 (H&E, 40x): Tumour budding BD score 2.

The correlation of tumor budding with various histopathological parameters were analysed in the present study. The most predominant Pattern of Invasion (POI) noted was Type 4 (n = 14, 35%). Out of these 14 cases, 13 cases had BD score 2 and only 1 case had BD score 1. All the cases of OSCC with Type 5 and one case of OSCC with Type 3 POI had BD score 2. Thus more number of tumor buds was seen in cases with worst pattern of invasion; however there was no statistically significant association between pattern of invasion and tumor budding in the current study (P-0.129). On correlating the other histopathological

parameters with tumor budding, statistically significant association was observed only with tumor (pT) stage (p-0.036) while there was no significant association with other variables like histological grade of OSCC (P- 0.36), lymph node metastases status (P- 0.23), tumor site (P-0.32), tumor size (P- 0.27), host lymphocytic response (P- 0.11), lymphovascular invasion (P- 0.5) and perineural invasion (P- 0.9) [Figure-2].

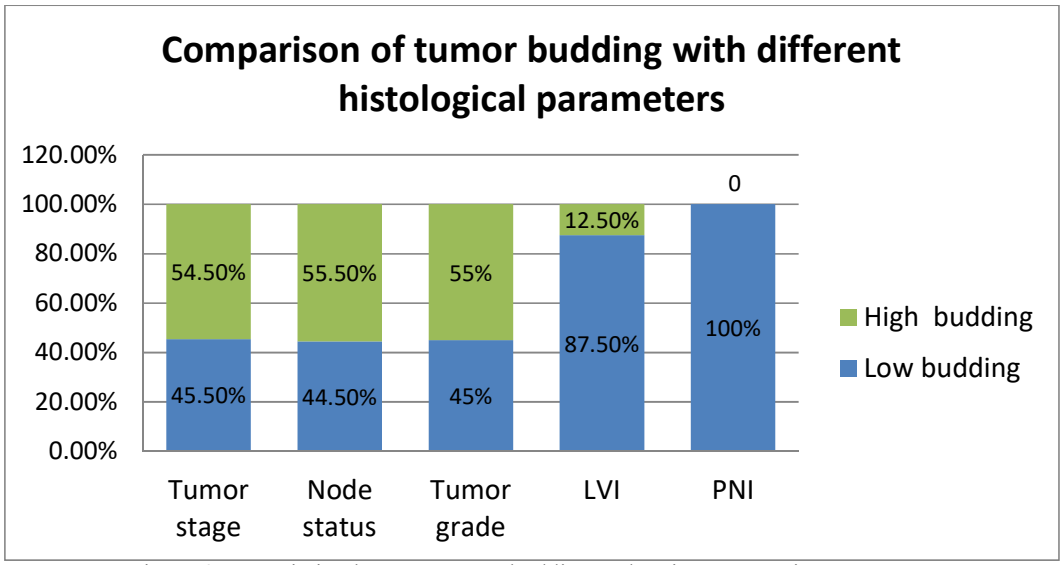

Figure-2: Association between tumor budding and major prognostic parameters.

DISCUSSION:

Tumor budding is defined as the presence of individual cancer cells or small clusters (fewer than five cancer cells) at the invasive front (IF) of the tumor and is considered as one of the important prognostic factors in gastrointestinal cancers. It is the first step in the metastasis of a solid tumor and is associated with Epithelial-Mesenchymal Transition (EMT).[1,2,5] Tumor budding combined with loss of cellular adhesion facilitates tumor invasion and metastasis at the invasive front. Tumor Buds show the differential expression of several EMT-related genes, including β-catenin, laminin-5 and claudins in cancer cells.[6] In oral quamous cell carcinomas, the expression of E-cadherin is significantly reduced in cells located in the invasive tumor front and in cells located in tumor buds than in other areas.[1,2]

In the study of oral and head and neck squamous cell carcinomas by Seki H et al [7] tumor budding was identified as a good predictive factor for lymph node metastasis in squamous cell carcinoma of the tongue and floor of the mouth unlike the present study where there was no significant association with lymph node metastasis.

There are still some variations in scoring tumor buds, and standardized scoring system is essential for appropriate comparison of results in head and neck squamous cell carcinomas. Many studies have reported that a high tumor budding score at the invasive front was strongly associated with cell proliferation, advanced stage and disease-specific mortality in OSCC similar to the present study.[8-12] Moreover, it was reported that tumor budding in OSCC correlated with locoregional recurrence and distant metastasis.[13-15] These findings suggest that despite the lack of a standardized scoring system, tumor budding is a reliable marker of tumor progression and adverse outcomes independent of the evaluation system used.

Intra-tumoural budding was reported as a valid method in colorectal cancer and only few studies have evaluated the prognostic value of tumour budding in invasive front of OSCC.[1,8] However, the tumor invasive front area might not be included in a biopsy specimen and in such cases, the intra-tumoural budding (tumour budding between tumour islands) might be more applicable.[16-17]

Future research on OSCC should compare the different evaluation methods with the goal of standardising the assessment method for histopathology reports. In addition, understanding the genetic basis of tumour budding may facilitate identification of treatment targets in OSCC. Even with all the limitations in this study, based on the findings, it is recommend that tumor budding evaluation should be included in routine histopathology reporting of OSCC.

CONCLUSION:

Association between budding and TNM stage supports the prognostic value of tumor budding in early OSCC and risk stratification. It is an easy, simple, reliable, reproducible and universally applicable prognostic factor that should be included in the histopathological reporting to predict prognosis of patients with OSCC after formulating a standardized scoring method.

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Conflicts of interest: There are no conflicts of interest.

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