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Prognostic value of tumor budding in oral squamous cell carcinoma

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ABSTRACT

Tumor budding is the histological phenomenon seen as a detached, discohesive small cluster of cells in the invasive tumor front. Tumor buds may provide a histological means for the assessment of epithelial-mesenchymal interaction and facilitate early prediction of prognosis.The present study aims to assess the prognostic significance of the tumor budding in oral squamous cell carcinoma(OSCC). Detailed Histopathological scoring of the oral squamous cell carcinoma tissue and lymph nodes including the evaluation of tumor budding was performed by two individual observers retrospectively. The association between various clinicopathological parameters and the budding index was analyzed using chi-square test and fisher's exact tests. Tumor budding was demonstrated in the invasive tumor front of all OSCC cases. High-intensity tumor budding was seen in stage 4 cancers with tumor size of > 4cms. Histologically, they were related to deep and extensive tumors, having an invasive front with infiltrative cells in groups or cords, dense stromal type in association with mild inflammation invariably. All of the cases with lymph node metastases were tumor budding positive. Thus this study emphasizes the importance of tumor budding evaluation in regular pathology practice in the management of OSCC cases.

Key Words

Prognosis, Oral Squamous Cell Carcinoma, Tumor budding, Histological evaluation

Introduction

Two-thirds of cancers occurring in India are Oral Cancers. Oral cancer commonly oral squamous cell carcinoma remains to have a poor five-year survival rate due to its early tendency to metastasize lymph nodes and frequent recurrences. Early prediction of oral cancer prognosis can help deliver appropriate treatment that does not underestimate the disease process.

The most progressed layers of tumor reside in the invasive tumor front, which forms the advancing edge of the cancer and gives excellent details of prognosis. " Tumor budding" is the histological phenomenon seen as a detached single or cluster of tumor cells in the invasive tumor front(1). These cells undergoing epithelial-mesenchymal transition has been well associated with high tumor grade, deep invasion, nodal metastasis, increased risk of recurrence and poor

prognosis in cancers of stomach, colorectal, pancreas, lung, breast and head & neck cancers including esophageal, nasopharyngeal and laryngeal carcinoma(2). Even though tumor budding is detected frequently in oral squamous cell carcinoma, its role as histological predictive marker has not been explored until recently. This simple, cost effective method can be of great value as a routine methodology of prognosis assessment in OSCC.

The present study aims to assess the prognostic significance of the tumor budding by assessing its association with clinicopathological parameters in oral squamous cell carcinoma.

Materials & Methods

Ethical approval was obtained from the institution standard review board prior to the commencement of the study. Eleven patients who had undergone excision of the primary lesion along with radical neck dissection for oral squamous cell carcinoma were retrospectively included in the study from the period of January 2017 to December 2017. The clinical details of the patient were collected from the biopsy requisition form and digital record management system central to the hospital. The details obtained include age, sex, presence/absence of habits, site, tumor size and TNM staging.

The excisional specimens of oral squamous cell carcinoma had been routinely fixed in 10% neutral buffered formalin, processed and paraffin embedded. 2-3 um paraffin sections were prepared and stained with hematoxylin and eosin. Various histological parameters including the presence and extent of tumor budding at the invasive tumor front of the OSCC lesions were evaluated by two independent pathologists separately using an observer score sheet. The parameters assessed in the study are summarized in the table 1.

To evaluate tumor budding, Slides were scanned under 4x first to select five areas displaying good intensity of budding. Then these 5 selected fields were counted under 10x to score the tumor budding intensity/budding index(3). Cases with 10 tumor buds were grouped into high intensity tumor budding, <10 tumor buds into Low intensity group and cases with <5tumor buds were considered negative.

Data Analysis

The data collected were tabulated and the association between clinicopathological parameters and the budding index was assessed using chi-square test and fisher's exact tests. The Interobserver agreement on various parameters scored was assessed using Kappa statistics. Statistical significance was set to the p value of <0.05. All the statistical analysis was performed using IBM SPSS statistics software version 21, United States.

Results

The study comprised eleven patient specimens who had undergone excision for oral squamous cell carcinoma. The results obtained are summarized in Table 2. Of the 11 patients included in the study 9 patients were male (81.8%) and 2 patients were female (18.2%). The mean age was 47.82 year (range from 30-65 years). The most common site was buccal mucosa (81.8%)

followed by mandibular (9.1%) and maxillary alveolus (9.1%). Most of them had the history of pan chewing/smoking/arecanut chewing /alcohol with a mean duration of 15 years. All of the patients included were in TNM stage 3 (45.5%) and stage 4 (54.5%). The tumor size of patients ranged from 2 to 5 cm with the majority of them in the T2 group (45.4%) followed by T3 (27.3%) and T4 (27.3%). All of the patients were clinical nodal metastasis positive with N1 (63.6%) followed by N2 (36.4%) stage.

Histological evaluation of the depth of invasion revealed that all of the tumors demonstrated deep or extensive invasion (100%). The histologic grading or differentiation at the invasive front showed 6 well differentiated tumors (54.5%) and 5 moderately differentiated tumors (45.5%). The most common type of invasive front encountered was tumor edge with Small groups or cords of infiltrative cells /nests (63.6%). Most tumors demonstrated either a dense stroma (36.4%) with mild inflammation (63.6%). Lymphovascular invasion was present in 1 tumor (9.1%) and perineural invasion was absent in all of the tumors. Histological examination of all levels of lymph nodes of radical neck specimen revealed presence of lymph node metastases in 4 cases (36.4%) while the rest were negative (7 cases, 63.6%)

The tumor buds were seen at the advancing edge of invasive tumor front in standard hematoxylin and eosin-stained sections. All of the 11 cases demonstrated tumor budding (100%) in the stroma. Of the 11 cases, 9 showed low-intensity tumor budding (<10 tumor buds) and 2 cases showed high-intensity tumor budding (>10 tumor buds). Excellent agreement was obtained for inter-observer rating for evaluation of tumor budding based on kappa statistics (k= .744).

All the clinical and histological parameters were cross tabulated individually against tumor budding. All the High-intensity tumor budding was seen in older age groups 40 years age, males, buccal mucosa cancers and stage 4 cancers with tumor size of > 4cms. Histologically, they were deep and extensive tumors, having an invasive front with infiltrative cells in groups or cords, dense stromal type in association with mild inflammation invariably. High tumor budding was seen in one of two cases with lymph node metastases. In the present study, only one case was lymphovascular invasion positive and was associated with low intensity bud score.

Discussion

Oral squamous cell carcinoma is a heterogeneous disease with high variations in treatment response and prognosis. Detailed histopathological grading with scoring of specific features can help individualize treatment and predict prognosis. Tumour budding is a histopathological phenomenon that symbolizes cellular dissociation and invasion. These cells are aggressive with maximal atypia thus contributing to the biological aggressiveness of the cancer. It's been an established prognostic feature in colon, rectum, esophageal including tongue cancers. Even the grading system incorporating budding and depth of invasion (8) had demonstrated good prognostic significance(4).

Although studies had indicated evaluation of tumor budding by using markers such as cytokeratin and EMT markers[5][2][6][2], tumor budding can easily be identified in H&E with good objectivity. In the present study, we have used only routine hematoxylin and eosin slides to

evaluate and score tumor buds into high intensity and low intensity. Grading criteria proposed by Ueno et al was used which had been reported to have more objectivity and good inter observer agreement(1)(3)(1). The statistical significant association between Low, High grades of tumor budding and various clinicopathological parameters in the study could not be achieved because of the small sample size and as the cross-tabulation was against the tumor budding grades and diverse sub-criterias in each variable.

All the tumors selected (100%) had an extensive depth of invasion (tumor thickness³4cms) and concomitantly showed presence of tumor budding. Depth of invasion has been demonstrated as a reliable parameter for predicting regional node involvement and patient survival in OSCC(7). It was significantly correlated with tumor budding in some of the previous studies (8)(1)(8). Increased depth of invasion by neoplastic growth induces molecular events that produce isolated tumor cells that break apart from the main tumor as tumor buds. Extensive invasion depth also determines close proximity of tumor buds to wider blood vessels & lymphatics present in deeper tissue making it less difficult for the tumor emboli to form in these structures*(7)*.

Worst pattern of invasive tumor front (WOPI), with small groups or cords of infiltrative cells /nests (63.6%) and dense stromal type was observed in association with tumor budding in most of the OSCC cases. The WOPI was previously also strongly associated with mortality from oral tongue SCC(8). Invasive patterns with cohesive cells (collective cell invasion) show good prognosis than those with discohesive cells that penetrates into normal surrounding tissues and acquires increased capacity (undergo EMT changes) for migration and invasion on exposure to increased levels of pro migratory signal molecules and a microenvironment produced by cells in stroma(9)(10)(9).

55.6% of low intensity and 100% of High intensity tumor budding was found to be associated with mild inflammation. Inflammatory cells infiltrate, considered as a result of tumor host interaction, activate cellular pathways by the chronic inflammation that can restrict the initial cancer cell transformation and progression(2). A previous study has demonstrated dense lymphocytic host response to produce complete response to therapy for oral tongue SCC(8). Absence or minimal inflammation favors the restriction free tumor invasion through tumour bud formation.

All the two OSCC cases with lymphovascular invasion were tumor budding positive. Whereas all of the tumor budding cases did not have lymphovascular invasion suggesting that tumor budding precedes and the isolated tumor buds acquire the cell motility to invade the vasculature and lymphatics effecting the metastasis process.

All of OSCC cases with cervical lymph node metastases showed tumor budding. Cervical lymph node metastasis is one of the major prognostic indicators in OSCC associated with decreased survival rates and recurrences. Many studies have postulated significant association with lymph node metastasis and histopathological parameters including high intensity tumor

budding(11)(12)(4)(13)(8)(12). In the present study, four of the cases showed lymph node metastasis when all of the cases showed tumor budding suggesting that tumor budding can be well recognized before lymph node invasion and thus serves a good prognosis marker.

Conclusion

Tumor budding is the frequently observed histological feature in OSCC. It was correlated with deep invasion, the worst pattern of invasive front along with minimal host response conditions in OSCC. Precedence of lymph node metastasis by tumor budding suggests it to be a good prognostic indicator. Thus this study emphasizes the importance of tumor budding evaluation in regular pathology practice in assessing the prognosis of OSCC cases.

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Tables and Figures

Table 1: Clinical and Histological parameters evaluated in the study.

Variables
1. **Age** <40 years/ >40 years
2. **Sex** Male/ Female
3. **Site** Buccal mucosa/Maxilla/Mandible/Tongue/ Gingiva/others
4. **Tumor size** PT_1: <2cm/ PT_2: >2cm and <4cm/ PT_3: >4cm/ PT_4: Advanced disease
5. **TNM Stage** 1/2/3/4
6. **Habits** Yes/No
7. **Depth of Invasion**[13] (measured from surface of tumor to deepest point of invasive tumor in paraffin- embedded sections) Early Carcinoma (<1mm) Invasion involving lamina propria (2.1- 4mm) Extensive and deep invasion (> 4mm)
8. **Histological grading** Well differentiated/Moderately differentiated/ Poorly differentiated
9. **Type of invasive tumor front** Pushing well-delineated borders Infiltrative solid cords/bands/strands Small groups or cords of infiltrative cells (nests)
10. **Stroma type** None / Delicate/ Dense/ Abundant

11. **Extent of inflammation** None /Slight/ Moderate/Marked
12. **Lymphovascular invasion** Absent / Present
13. **Lymph node metastasis** Absent / Present (Extra capsular / Intra capsular)
14**. Perineural invasion**Absent / Present

Table 2: Tumor budding and its association with the clinicopathological variables (Chi-square and Fisher's exact test)

Variable	**No of patients (N=11)**	**Low-intensity tumor budding (n=9)**	**High-intensity tumor budding (n=2)**	**P value**
Age (in yrs.) <40 >=40	1(9.1%) 10 (90.9%)	1(11.1%) 8(88.9%)	0 2(100%)	.818
Sex Male Female	9(81.8%) 2(18.2%)	7(77.8%) 2(22.2%)	2(100%) 0	.655
Site Buccal mucosa Maxilla Mandible	9(81.8%) 1(9.1%) 1(9.1%)	7(77.8%) 1(11.1%) 1(11.1%)	2(100%) 0 0	.762
Tumor size PT_1: £2cm PT_2: >2cm and <4cm PT_3: ³4cm PT_4: Advanced disease	0 5 (45.4%) 3 (27.3%) 3 (27.3%)	0 5(55.6%) 2(22.2%) 2(22.2%)	0 0 1(50%) 1(50%)	.361

Stage 1 2 3 4	 0 0 5(45.5%) 6(54.5%)	 0 0 5(55.6%) 4(44.4%)	 0 0 0 2(100%)	 .154
Habits No Yes	 10(90.9%) 1(9.1%)	 1(11.1%) 8(88.9%)	 0 2(100%)	 .621
Depth of Invasion 1. Early Carcinoma 2. Invasion involving lamina propria 3. Extensive and deep invasion	 0 0 11(100%)	 0 0 9(100%)	 0 0 2(100%)	 0
Histological grading 1. Well differentiated 2. Moderately differentiated 3. Poorly differentiated	 6(54.5%) 5(45.5%) 0	 5(55.6%) 4(44.4%) 0	 1(50%) 1(50%) 0	 .727
Type of invasive front 1: Pushing well-delineated borders 2: Infiltrative solid cords/bands/strands 3: Small groups or cords of infiltrative cells (nests)	 4(36.4%) 0 7(63.6%)	 4(44.4%) 0 5(55.6%)	 0 0 2(100%)	 .382

Stroma type 1. None 2. Delicate 3. Dense 4. Abundant	4(36.4%) 3(27.3%) 4(36.4%) 0	4(44.4%) 3(33.3%) 2(22.2%) 0	0 0 2(100%) 0	.118
Extent of inflammation 1: None 2: Slight 3: Moderate 4: Marked	1(9.1%) 7(63.6%) 2(18.2%) 1(9.1%)	1(11.1%) 5(55.6%) 2(22.2%) 1(11.1%)	0 2(100%) 0 0	.706
Lymphovascular invasion 0: Absent 1: Present	10(90.9%) 1(9.1%)	8(88.9%) 1(11.1%)	2(100%) 0	.818
Lymph node metastasis 0: Absent 1: Present	7(63.6%) 4(36.4%)	6(66.7%) 3(33.3%)	1(50%) 1(50%)	.618
Perineural invasion 0: Absent 1: Present	0 0	0 0	0 0	-