Tumour Budding and Depth of Invasion as Predictors of Occult Cervical Lymph Node Metastasis in Early Oral Squamous Cell Carcinoma

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Abstract
The purpose of this study was to determine the importance of detection of metastatic disease in the cervical lymph nodes in early oral squamous cell carcinoma (OSCC). The challenge of this is to be able to identify those clinically node-negative (cN0) patients who actually have that hidden risk and hence might benefit from elective neck dissection which requires reliable pathological indicators.
The aim of this investigation was to verify the relationship between tumour budding and depth of invasion (DOI) and with occult cervical lymph node metastasis (OCLNM) in early stage OSCCs.
Methods: A total of sixty-two patients with early stage (cT1-T2N0) OSCC who underwent primary tumour resection followed by elective neck dissection were followed up in a retrospective fashion. All the age, sex, location of the tumour, histological grade, DOI, tumour budding score and nodal status were collected from the chart review of each case. The budding was assessed on regular H&E sections as per the guidelines of ITBCC adapted for OSCC, and the DOI was assessed based on the AJCC 8th edition guidelines. SPSS v30.0 (IBM) was used for statistical analysis: categorical associations were tested using Chi-square or Fisher's exact test, and the multivariable binary logistic regression was used to identify independent predictors of occult nodal metastasis with a α value of <0.05.
Results: Eighteen of the 62 patients (29.0%) turned out to have occult nodal metastasis. This correlated significantly with tumour stage ($p=0.047$), tumour grade ($p=0.038$), DOI $\geq$5 mm ($p=0.006$) and a high budding score ($p<0.001$). The average DOI was significantly greater in node-positive patients (8.1 $\pm$ 2.3 mm) versus node-negative patients (4.9 $\pm$ 1.8 mm; $p<0.001$). In the regression model, both high budding (OR=6.84; 95% CI 1.95–23.96; $p=0.003$) and DOI $\geq$5 mm (OR=4.27; 95% CI 1.21–15.08; $p=0.024$) stood as independent predictors.
Conclusion: DOI and tumour budding are independent markers of occult metastatic risk in early OSCC. Incorporating them into routine pathological evaluation could be useful for refining risk categorization and for better selection of patients for elective neck dissection.
Keywords: Tumour budding, Oral squamous cell carcinoma, Depth of invasion, Elective neck dissection, Occult lymph node metastasis, Prognostic marker, Histopathology.
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Introduction

OSCC is by far the most common oral malignancy worldwide, accounting for almost 90% of all oral cancers. Despite the advances in diagnostics and treatment, the status of the cervical nodes to date remains the most important determinant of the risk of recurrence, the extent of treatment and survival rates. However, a significant proportion of early-stage (cT1-T2N0) disease are clinically silent and still have unsuspected spread to the neck which frequently poses a treatment challenge. [2,4]
No consensus on management of cN0 neck in early OSCC. Whilst it is obvious that elective dissection is likely to benefit patients with undetected nodal disease, it may have the potential to put phenotypically low risk patients through a needless surgery and its risks. It is this tension that makes histopathological tools to stratify risk important for individualising treatment. [2–5]
Of the markers, how far the tumour cells project beyond the basement membrane (after all of them are outside), known as the DOI, has been one of the more reliable markers and is always associated with regional metastasis, local recurrence, and shorter survival. The introduction to the 8th edition AJCC staging system now recognizes it as a true prognosticator and stage determinant. [3–6]
Tumour budding, which is more recent, on the other hand, has become a more accepted tumour aggressiveness marker. Histologically characterized as individual cells or small clusters of up to 5 cells at invasive edge; considered to be a morphological

characteristic of epithelial–mesenchymal transition, which is associated with enhanced invasive and metastatic activity. There is presently a continuous string of investigations that have associated high budding scores to lymphovascular invasion, perineural spread, nodal metastasis, recurrence and reduced survival in OSCC. [1,5–9]

Reviewing budding and DOI simultaneously provides the most comprehensive outlook for the two. Both are simple enough to be inexpensive, reproducible and realistic when used in daily use, and this is important for anything that would be considered to help select patients for elective neck dissection and guide the surgeon in his or her decision making process. [6–10]

It was this background that led us to design this study, which aimed at evaluating the correlation between tumour budding, DOI and clinically undetectable cervical nodal metastasis and evaluating the predictive power of these two histopathological parameters to categorize high-risk patients with occult cervical nodal metastasis for evidence-based management in early OSCC. [1–10]

Methodology

In this retrospective study, the researchers spent two years interviewing patients from a tertiary teaching hospital with the approval of the Institutional Ethics Committee. The cohort consisted of 62 patients with confirmed early OSCC (cT1–T2N0) seen during the period and the sample did not involve any selection process, merely including any eligible patient who presented with OSCC during the study period.

The inclusion criteria were adult patients (aged ≥18 years) who were diagnosed with cN0 OSCC of the oral cavity with complete clinicopathological data. The cases were excluded if there was recurrence of the tumour, previous chemo- or radiotherapy, distant metastases at diagnosis, a second primary cancer, inadequate specimen for pathological analysis, incomplete records or previous neoadjuvant treatment.

Age, sex, site of lesion, tumour size, grade, DOI, budding score and nodal status were available on charts and pathology reports. Each H&E slide was independently reviewed by two oral pathologists who were both unaware of the nodal outcome and in cases of disagreement, a joint re-examination was performed with consensus reached.

The distance from the baseline of the adjacent mucosa to the deepest point of invasion of the tumour was measured by the ocular micrometre under light microscopy and was classified according to the AJCC 8th-edition system.

The invasive front was scanned at a low magnification (×10) for identification of the densest budding area (hotspot) containing less than 5 tumour cells or a single cell and then budding counts were made in this area at ×20 magnification, according to the modified ITBCC 2016 protocol adapted for OSCC. Prior to the OSCC studies, counts less than 5 were considered low budding and counts more than 5 were considered high budding. Several neck dissection specimens confirmed the presence of small volume, occult N1 disease histopathologically.

The primary analysis focused on the correlation between budding and DOI and the secondary analysis was performed to evaluate the relationship between budding and DOI with age, sex, tumour site, stage and grade.

All data were recorded in excel and analysed using SPSS v30.0 (IBM, Armonk, NY). Data on continuous variables are presented as means ± standard deviations and data on categorical variables are given by numbers and percentages. Parametric and non-parametric (t-test versus Mann–Whitney U) comparisons were chosen for Shapiro–Wilk testing; categorical comparisons were made with Chi-square or Fisher's exact test as appropriate. All univariately significant variables subsequently served as independent variables in a multivariable binary logistic regression model to identify independent factors predictive of occult metastasis; two-tailed p values <0.05 were considered significant throughout.

Results

The analysed group consisted of sixty two early OSCC patients (cT1–T2N0) who underwent surgery and elective neck dissection surgery. As Table 1 shows, the cohort averaged 52.8 ± 11.6 years old, skewed toward patients 50 or older (61.3%) and male (67.7%). The most common tumour sites were buccal mucosa (38.7%) and tongue (32.3%) and the most common tumour stages were T2 (58.1%) and well-differentiated (62.9%). DOI hit ≥5 mm in 69.4% of cases, and 46.8% showed high budding. FNA was positive in 19 cases (34.2%), 18 of whom (29.0%) were found to have FNA missed disease, that is, occult metastasis.

Table 2 breaks down which clinicopathological factors tracked with occult metastasis. Age ($p=0.593$) and sex ($p=0.741$) showed no meaningful link, but tumour stage ($p=0.047$), grade ($p=0.038$), DOI ($p=0.006$), and budding ($p<0.001$) all did. A greater invasion depth (geq 5 mm) and a higher the budding score were both associated with significantly higher occult-metastasis rates.

Table 3 compares the two: 8.1 ± 2.3 mm for the node-positive patients and 4.9 ± 1.8 mm for the node-negative ($p<0.001$) — a distance that is designed to emphasize the close relationship between the depth of the tumour and the presence of nodes.

When the univariately significant factors were then taken into logistic regression, high budding proved as the one strongest independent factor (OR 6.84; 95%

CI 1.95–23.96; p=0.003), and DOI ≥5 mm close behind (OR 4.27; 95% CI 1.21–15.08; p=0.024). Stage and grade were univariate significant, but were not seen to survive multivariable adjustment (Table 4).

In other words, nearly 33% of this early OSCC group had nodal disease that was not observed by their physicians. When all parameters were evaluated, budding and DOI independently and most consistently predicted these findings, suggesting their use for routine pathological assessment of primary disease, especially regarding management of a clinically negative neck..

Table 1. Demographic and Clinicopathological Characteristics of the Study Population (n = 62)

Variable	Category	n (%)
Age (years)	Mean ± SD	52.8 ± 11.6
Age group	<50 years	24 (38.7)
	≥50 years	38 (61.3)
Sex	Male	42 (67.7)
	Female	20 (32.3)
Tumor site	Buccal mucosa	24 (38.7)
	Tongue	20 (32.3)
	Floor of mouth	8 (12.9)
	Gingivobuccal sulcus	6 (9.7)
	Others	4 (6.4)
Tumor size	T1	26 (41.9)
	T2	36 (58.1)
Histological grade	Well differentiated	39 (62.9)
	Moderately differentiated	20 (32.3)
	Poorly differentiated	3 (4.8)
Depth of invasion	<5 mm	19 (30.6)
	≥5 mm	43 (69.4)
Tumor budding	Low (<5 buds)	33 (53.2)
	High (≥5 buds)	29 (46.8)
Occult cervical lymph node metastasis	Present	18 (29.0)
	Absent	44 (71.0)

Table 2. Association of Clinicopathological Variables with Occult Cervical Lymph Node Metastasis

Variable	Metastasis Present n=18	Metastasis Absent n=44	p-value
Age <50 years	8	16	0.593
Age ≥50 years	10	28	
Male	13	29	0.741
Female	5	15	
T1	4	22	0.047*
T2	14	22	
Well differentiated	8	31	0.038*
Moderate/Poor	10	13	
DOI <5 mm	2	17	0.006*
DOI ≥5 mm	16	27	
Low tumor budding	3	30	<0.001*
High tumor budding	15	14	

*Chi-square/Fisher's Exact Test

Table 3. Mean Depth of Invasion According to Cervical Lymph Node Status

Lymph node status	Mean DOI (mm)	SD	p-value
Metastasis Present	8.1	2.3	<0.001
Metastasis Absent	4.9	1.8	

Independent Student's t-test

Table 4. Multivariable Binary Logistic Regression Analysis for Predictors of Occult Cervical Lymph Node Metastasis

Variable	Odds Ratio (OR)	95% CI	p-value
High tumor budding	6.84	1.95–23.96	0.003*
DOI ≥5 mm	4.27	1.21–15.08	0.024*
T2 stage	1.89	0.58–6.11	0.287
Moderate/Poor grade	2.16	0.71–6.58	0.173

Discussion

The cN0 neck in early OSCC is indeed a grey area due to the high significance of "occult" nodal metastases on recurrence and survival. That decision is feasible thanks to dependable histopathological predictors and the finding of this study—both tumour budding and DOI have a meaningful indication of nodal involvement.

The incidence of occult-metastasis reported here of 29.0% was well within the range of 20 to 35% reported elsewhere for the incidence of occult-metastasis in clinically node-negative early OSCC. Fukano's group also correlated the risk of metastasis

in the neck with the depth of the tumour and the same conclusion was reached in the study conducted by Kurokawa and Sakai as well, both indicating that DOI is a real prognostic and predictive factor. [2,3,4]

In this series, obviously the metastasis signal of DOI crossing 5 mm, and the average depth of invasion of the node-positive patients was significantly deeper than that of the node-negative. This is consistent with recent systematic reviews where DOI has emerged as an independent risk factor for nodal spread, recurrence, and worse survival in early OSCC which contributed to its inclusion in the 8th edition of AJCC. [6]

It is perhaps the other of the two markers that has become the more discussed in terms of a measure of tumour aggressiveness, and that is budding. Occult-metastasis rate was also markedly higher in this cohort of high-budding patients (≥5 buds) than in low-budding ones, and budding was the strongest independent predictor in the multivariate modelling performed in this study, confirming Shimizu's findings that high budding is associated with nodal involvement, recurrence, and reduced disease-free survival, and Xie's earlier work that budding is an independent predictor of OSCC, overall. [1,7,8]

Mechanistically, budding is often thought to represent a phenological manifestation of epithelial to mesenchymal transition (EMT), where tumour cells lose adhesive contacts, gain motility and break into surrounding tissues, which corresponds to an increased invasive and metastatic potential. This is echoed by the studies of Binmadi and Mohamed, who noted an increase in poor features with an increase in number of buds – lymphovascular invasion, nodal metastasis, perineural spread etc. [6,7,9,12]

The important thing to note about this dataset was that budding and DOI maintained their predictive power even when they were added with the other variables, while the other two factors (stage and grade), which proved important on their own, disappeared once added with the other factors. The difference in nuclear attributes indicates that conventional grading does not reflect the complete aggressiveness of the tumour, whereas budding and DOI reflect a more objective measurement of the aggressiveness — which is precisely what Verma's group and Srinivasan and Balasubramaniam have independently concluded: that these two markers are superior to grading alone. [5,10]

Clinically, the combination of budding and DOI appears to be a feasible method for risk stratification of early OSCC patients. They require nothing more than the standard H&E slide, which are not an extra expense or technical burden, but simply a reproducible, clinic ready, pair of biomarkers. Others have already called for the adoption of budding as a regular parameter of reporting because the presence of budding should mean that the patient may be referred for elective neck dissection when he or she may not have been without budding. [5,10,15]

There are limitations to this study. The single-centre retrospective design and relatively small cohort size limit generalisability of the results, while absence of long-term recurrence and survival data precluded assessing the prognostic significance of budding and DOI in real-life outcomes. Large, multi-centre prospective studies with sufficient follow-up time to substantiate these findings and to establish standardized budding cutoffs for oral SCC in particular will be required.

None of that detracts from the main message, however: the routine assessment of budding and DOI in early OSCC appears to be well justified by this data, as both proved to be strong predictors of occult nodal disease, and were good independent predictors. Incorporation into routine reports by the pathologist could actually aid the clinical decision making of who will most benefit from elective neck dissection.

Conclusion

Tumour budding and DOI ≥5 mm were found to be important independent predictors of the presence of occult cervical nodes in early stage OSCC, tumour budding proving the more significant of the two. Since both are easily assessed at ordinary H&E sections and there's no extra cost involved, incorporating them into routine pathology reports is a pragmatic approach to improved risk categorization and neck dissection decisions. Larger prospective studies in multiple centres - which confirm and extend these findings - will ultimately support standardised advice for the clinical setting..

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