Original Article

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Pathology Section

Tumour Budding as a Predictive Factor for Lymph Node Metastases in Preoperative Oral Cancer Biopsies: A Retrospective Study

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ABSTRACT

Introduction: The enormous advancement in understanding of Oral Squamous Cell Carcinomas (OSCCs) has not been accompanied by a significant reduction in the high morbidity and mortality rates associated primarily with disease recurrence and lymph node metastases.

Aim: To evaluate the tumour budding as an independent prognostic marker to predict lymph node metastasis in preoperative biopsies.

Materials and Methods: The present retrospective study was conducted in the Department of Pathology, Guntur Medical College (tertiary care centre), Andhra Pradesh, India, from January 2018 to December 2021. Samples was collected from 32 patients with preoperative diagnostic oral cavity biopsies, who also underwent resection with cervical lymph node dissection. The degree of differentiation, preoperative tumour budding, postoperative tumour budding, and intratumoural budding in preoperative biopsies were all assessed histologically in each case. The results were analysed using Chi-square test, Kaplan-Meier method and log-rank test.

Results: Thirty two OSCC cases were examined by Haematoxylin and Eosin (H&E) stained slides. By using univariate analysis, the histological factors like depth of invasion (p-value=0.04), pattern of invasion (p-value=0.004), presence of preoperative tumour budding (p-value=0.008), postoperative tumour budding (p-value=0.004), intratumoural tumour budding (p-value=0.019), and sex (p-value=0.03) all significantly associated with risk of lymph node metastasis. Other clinical and histological factors, including age, the largest tumour size, histological grade, Lymphovascular Invasion (LVI), Perineural Invasion (PNI), and stromal response, did not significantly associate with the probability of lymph node metastasis.

Conclusion: The potential of morphological features, such as Tumour Budding (TB) evaluated in OSCC diagnostic preoperative biopsies may aid in identifying patients who may benefit from more aggressive treatments.

Keywords: Buds, Small biopsy, Squamous cell carcinoma, Tongue

INTRODUCTION

According to GLOBOCAN, oral cavity carcinoma is the eighth most prevalent cancer in the world, with an expected 354,900 new cases in 2018. Of these, 90% are Oral Squamous Cell Carcinoma (OSCC), which have a 60% 5-year survival rate [1]. The Tumour Node Metastasis (TNM) staging system is used to determine prognosis and stratify patients into management schemes. Enormous amounts of molecular studies have been done in OSCC to identify biomarkers that can predict prognostic outcomes; however, none of them have shown convincing results with ambiguous results and insufficient evidence regarding their usefulness, precluding their use in routine practice. The search for a reliable prognostic parameter continues. If identified, the elusive parameter would improve patient categorisation based on tumour aggressiveness and guide more effective and personalised therapeutic options. Tumour budding is a widely studied prognostic parameter in colorectal cancers [2].

The H&E stained sections are typically used to assess a variety of histopathologic prognostic criteria, such as tumour grade, depth of invasion, Perineural Invasion (PNI), Lymphovascular Invasion (LVI), lymphocytic host response, and mitotic activity. These details are presented in pathology reports to help how OSCC may behave. This is crucial for designing an effective and efficient management. However, some of these factors (such as tumour grade and lymphocytic response), particularly in early-stage OSCC, have not proven to be reliable prognostic indicators [3,4]. Additionally, a number of new biomarkers for OSCC have been discovered in recent studies, but they are not yet acceptable for inclusion in the pathology report [5,6].

The majority of research has examined Tumour Budding (TB) using excisional specimens that clearly display the depth of the tumour tissue and make it simple to assess the invasive front. Seki M et al., investigated this measure in preoperative biopsy specimens and found a strong connection with tumour bud counts before and after surgery [5,6]. It should be highlighted that in order to clearly see the invasive front in preoperative biopsy specimens, the surgeons should do a big biopsy that includes the deepest area of the tumour, which is not always possible.

Consequently, there is still a real need for a prognostic metric that is more reliable and consistent. If the elusive metric were to be discovered, it would enable better patient classification based on the aggressive behaviour of the tumour and, in the end, serve as a guide for more efficient and individualised therapy alternatives. Tumour budding is one such crucial prognostic factor that has been extensively studied in colorectal carcinomas but is frequently characterised in many malignancies (TB) [2]. The current study's objectives were to examine tumour budding in OSCC and present its prognostic significance.

MATERIALS AND METHODS

The present retrospective study was conducted in the Department of Pathology, Guntur Medical College (tertiary care centre), Andhra

Pradesh, India, from January 2018 to December 2021. Study included patients of preoperative diagnostic oral cavity biopsies that also underwent resection with cervical lymph node dissection.

Inclusion criteria: Excision biopsy was done for 59 cases, of these lymph node dissection was done in 32 cases, which were included in the study. Lesions from the tongue and buccal mucosa were included in the study.

Exclusion criteria: All the patients for whom lymph node dissection was not done were excluded from the study.

Study Procedure

From the archive, all of the slides were taken out and examined. The degree of differentiation, preoperative tumour budding, postoperative tumour budding, and intratumoural budding in preoperative biopsies were all assessed histologically in each instance. Tumour stage was classified as T1, T2, T3 and T4 according to Tumour, Node and Metastasis (TNM) staging.

According to their level of differentiation, the tumours were also histologically categorised as well, moderately or poorly differentiated squamous cell carcinoma. PNI was defined as the tumour surrounding or invading a nerve. LVI was applicable to tumour invasion within lymphatic, venous, or arterial pathways. The loose, desmoplastic and hyalinised stromal response was rated. Pattern of invasion is divided into infiltrative or non infiltrative pattern. The distance between the lowest point of the surrounding healthy mucosa and the lowest point of the tumour is measured for the depth of invasion. Using a slide calliper, the depth was measured in millimetres and classified as D1 (5 mm), D2 (>5-10 mm), and D3 (>10 mm) [3]. A single tumour cell or a group of five or more tumour cells located in the stroma near the invasive tumour front was referred to as tumour budding and its presence and absence was assessed [3].

STATISTICAL ANALYSIS

Data were collected, revised, coded and entered to the Statistical Package for Social Sciences (SPSS) IBM software version 21.0. The qualitative variables were presented as number and percentages. The comparison between two groups was done by using Chi-square test, Kaplan-Meier method and log-rank test. The factors influencing the likelihood of Lymph Node (LN) metastasis were evaluated using a univariate analysis, to find independent predictors. Every test was two-sided, and p-value <0.05 was used to determine significance.

RESULTS

This was a retrospective study, and total of 114 preoperative oral cavity cancer biopsies were performed during the study period. Only the tongue and buccal mucosa were examined because the other parts were infrequently impacted. Thirty two patients of preoperative diagnostic oral cavity biopsies that also underwent resection with cervical lymph node dissection were included in the study for analysis. There were three females and 29 males with male to female ratio of 9.6:1. The patients were between the age group of 22 years and 80 years. The summary of all patients is shown in [Table/Fig-1]. Tumour budding was noted in 72% of excision specimen cases, 68% of preoperative small biopsy cases and 28% of preoperative small biopsy cases showed intratumoural tumour budding. There were 5 (16%) T1, 19 (59%) T2, 5 (16%) T3 and 3 (9%) T4 tumours. Eight (25%) of the carcinomas were well differentiated, 15 (47%) were moderately differentiated and 9 (28%) were poorly differentiated. Metastasis to the lymph nodes was seen in 18 (56%) cases. The margins of every case were tumour free.

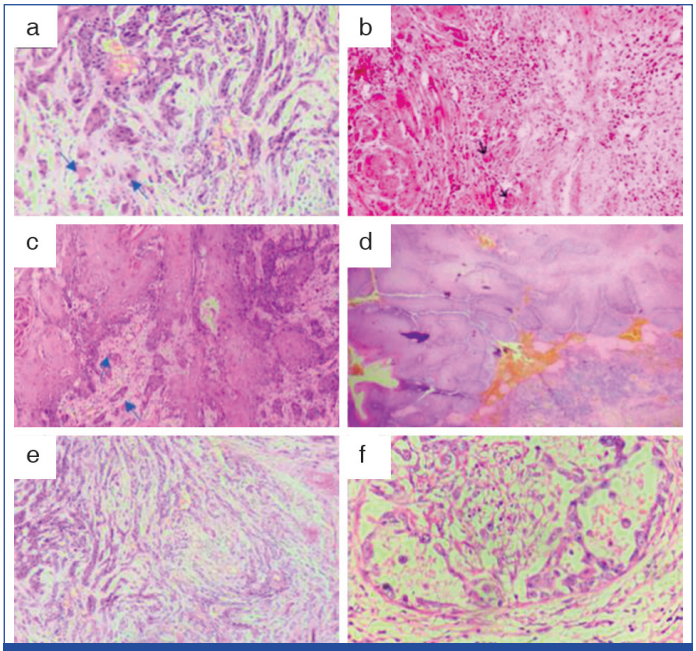
Tumour budding between preoperative biopsies [Table/Fig-2a] and postoperative specimens [Table/Fig-2b] was analysed, as well as

Parameters	n (%)
Age (years)	
<40	10 (31)
≥40	22 (69)
Sex	
Males	29 (91)
Females	3 (9)
Lymph node metastasis	
Present	18 (56)
Absent	14 (44)
Tumour budding- Excision biopsy	
Present	23 (72)
Absent	9 (28)
Tumour budding- Preoperative biopsy	
Present	22 (69)
Absent	10 (31)
Intratumoural tumour budding- Preoperative biopsy	
Present	9 (28)
Absent	23 (72)
Tumour grade	
Well differentiated	8 (25)
Moderately differentiated	15 (47)
Poorly differentiated	9 (28)
Tumour stage	
T1	5 (16)
T2	19 (59)
T3	5 (16)
T4	3 (9)
Pattern of invasion	
Non infiltrative	11 (34)
Infiltrative	21 (66)
Depth of invasion	
<5 mm	11 (34)
5-10 mm	15 (47)
>10 mm	6 (19)
Perineural Invasion (PNI)	
Present	10 (31)
Absent	22 (69)
Lymphovascular Invasion (LVI)	
Present	21 (66)
Absent	11 (34)
Stromal response	
Loose	20 (63)
Desmopastic	11 (34)
Hyalinised	1 (3)

[Table/Fig-1]: Clinical and pathological summary of all included cases in the study.

Intratumoural Tumour Budding (ITB) [Table/Fig-2c] in preoperative biopsies. Also, non infiltrative pattern of invasion [Table/Fig-2d], infiltrative pattern of invasion [Table/Fig-2e], LVI [Table/Fig-2f] depth of invasion, PNI, stromal response, tumour size were assessed and compared with lymph node metastasis positive and lymph node metastasis free cases, as shown in [Table/Fig-3].

Among all cases, clinical and pathological parameters were associated with lymph node metastasis as shown in [Table/Fig-3]. By using univariate analysis, the histological factors depth of invasion (p-value=0.04), pattern of invasion (p-value=0.004), presence of preoperative tumour budding (p-value=0.008), postoperative tumour budding (p-value=0.004), intratumoural



[Table/Fig-2]: a) Tumour buds (blue arrows) proliferating into the underlying connective tissue at the invasive front of oral squamous cell carcinoma preoperative biopsies (H&E stain, 400X); b) Tumour buds (black arrows) proliferating into the underlying connective tissue at the invasive front of oral squamous cell carcinoma in postoperative excision biopsy (H&E stain, 400X); c) Tumour buds (blue arrows) intratumoural budding (in-between the tumour cells) in preoperative biopsies (H&E stain, 400X); d) Tumour with pushing pattern of infiltration (H&E stain, 400X); e) Tumour with infiltrative pattern of invasion (H&E stain, 400X); f) Tumour cells attached to the endothelial lining of the vessel with RBCS (H&E stain, 400X).

Parameters	Lymph node free N=14 (43.8%) n (%)	Lymph node metastasis N=18 (56.2%) n (%)	p-value
Age (years)			
<40	4 (28)	6 (33)	0.770
≥40	10 (72)	12 (67)	
Sex			
Male	11 (79)	18 (100)	**0.030**
Female	3 (21)	0 (0)	
Tumour stage			
T1	3 (21)	2 (11)	0.390
T2	5 (36)	14 (77)	
T3	4 (29)	1 (6)	
T4	2 (14)	1 (6)	
Histological grade			
Well differentiated	3 (21)	5 (28)	0.250
Moderately differentiated	5 (36)	10 (56)	
Poorly differentiated	6 (43)	3 (17)	
Pattern of invasion			
Non infiltrative pattern	9 (64.3)	2 (11)	**0.004**
Infiltrative pattern	5 (35.7)	16 (89)	
Preoperative tumour budding			
Present	6 (43)	16 (89)	**0.008**
Absent	8 (57)	2 (11)	
Preoperative intratumoural budding			
Present	1 (7)	8 (44)	**0.019**
Absent	13 (93)	10 (56)	
Postoperative tumour budding			
Present	6 (43)	17 (94)	**0.004**
Absent	8 (57)	1 (6)	
Depth of invasion			
D1	8 (57)	3 (17)	**0.044**
D2	5 (36)	10 (55)	
D3	1 (7)	5 (28)	
Perineural Invasion (PNI)			
Present	4 (28)	6 (33)	0.770
Absent	10 (72)	12 (67)	
Lymphovascular Invasion (LVI)			
Present	9 (64)	12 (67)	0.888
Absent	5 (36)	6 (33)	
Stromal response			
Loose	11 (79)	9 (50)	0.160
Desmoplastic	2 (14)	9 (50)	
Hyalinised	1 (7)	00 ()	

[Table/Fig-3]: Association of lymph node metastasis with clinical and pathologic variables.
p-value in bold font represents statistically significant value. p-value calculated using Chi-square test, Kaplan-Meier method and log-rank test

tumour budding (p-value=0.019), and sex (p-value=0.03) all significantly associated with risk of lymph node metastasis. Other clinical and histological factors, including age, the largest tumour size, histological grade, LVI, PNI, and stromal response, did not significantly associate with the probability of lymph node metastasis in oral cancers.

DISCUSSION

In the cases of colon cancer, lung cancer, oesophageal cancer and pancreatic cancer, tumour budding has been confirmed as a promising prognostic sign [4]. Seki M et al., and Seki M et al., assessed tumour budding in preoperative biopsies and correlated it with the tumour bud count in postoperative specimens, and they found a good correlation which, is similar to the present study [5,6]. It has also been highlighted that if tumour budding is assessed in small biopsy specimens, the surgeons must perform a bigger biopsy that contains the deepest section of the tumour tissue in order to detect the tumour's invasive front, which may not always be possible. Preoperative tumour budding has been shown to have considerable predictive value for lymph node metastasis, overall survival, and disease-free survival in a recent systematic analysis. Therefore, it may be advantageous if we could use a tumour budding evaluation to forecast the tumour's aggressiveness before surgery and apply the results to therapeutic considerations [7].

Tumour bud count performed noticeably better than commonly employed measures including tumour size, grade, and depth of invasion in predicting lymph node metastasis, according to studies by Pedersen NJ et al., and Angadi PV et al., [8,9].

Peritumour Budding (Peri-TB) and ITB are terms used to describe TB that is present at the invasive front of the tumour, TB that is present in the tumour tissue, respectively [10-13]. ITB has not received much attention in the literature, but a number of authors have described bud like structures in the primary tumour mass of several malignancies, including colorectal, breast, and rectal [12,13]. Preoperative assessment of ITB, particularly in shallow/small biopsies, may serve as an important prognostic signal to be included in standard histopathological reporting since there is no study of ITB in OSCC.

Authors noted the relevant studies in accordance with the present study [Table/Fig-4] [5,6,14]. There were very few studies demonstrated tumour budding in preoperative biopsies of oral cancer.

A successful biopsy is necessary for the proper evaluation of TB. In a study, poorly differentiated tumour clusters of five or more cells were evaluated using the histopathological findings from atleast three preoperative biopsies [15]. However, severe specimen fragmentation, tangential biopsy effects, artefactual alterations, and the presence of widespread necrosis can all make it difficult to

Author and year of the study	Country	No. of cases	Bud definition	Type of tumour	Magnification field	Stain	Summary of study
Seki M et al., 2016 [5]	Japan	91	<5 cells	Tongue cancers	20X	Cytokeratin	Preoperative TB was significantly associated with LN metastasis (OR 31, p-value <0.01), OS and RFS (p-value <0.05)
Seki M et al., 2017 [6]	Japan	209	<5 cells	Oral squamous cell carcinoma	20X	Cytokeratin	Strong correlations (p-value <0.01) were observed between preoperative TB and tumour grade, tumour depth, INF and blood vessel invasion. Preoperative TB correlated with LNM (OR 30.05, p-value <0.01)
Almangush A et al., 2018 [14]	Finland	100	<5 cells	Oral tongue cancers	20X	H&E	There was a statistically significant relationship (p-value <0.001) between TB score in preoperative and postoperative samples
Present study, 2023	India	32	<5 cells	Tongue and buccal mucosa	20X	H&E	There was statistically significant relationship between preoperative TB, ITB, postoperative TB and pattern of invasion to LN metastasis.

[Table/Fig-4]: Studies that conducted tumour budding evaluation in preoperative diagnostic biopsies [5,6,14].

detect TB and lower the quality of pretreatment diagnostic biopsies. Surprisingly, intratumoural budding at the tumour's invasive border substantially linked with peritumoural tumour budding [16]. Since, it is frequently difficult to detect an invasive tumour front in diagnostic tiny biopsies to evaluate the TB at the invasive front of tumour in colorectal cancers [17].

Early analysis of 56 biopsy samples from OSCC cases revealed the potential significance of the relationship between histological grade, pattern of invasion and TB intensity [18]. Whereas in the present study, tumour grade did not show any significant relationship with lymph node metastasis, but tumour budding and pattern of invasion had significant relationship with LN metastasis. Leite CF et al., divided the pattern of invasion into four degrees and TB as low and high-intensity which is similar to proposal by Shimizu S et al., study [18,19]. The majority of the cases under study (66.1%) had high intensity TB with the worst form of invasion, although there was no correlation between the two. The authors came to the conclusion that patients who would benefit from aggressive therapy might be chosen using both the pattern of invasion and TB in diagnostic specimens. These findings therefore call for additional research using a bigger sample size and longer follow-up.

An assessment of the microenvironment in early malignancies may be possible with a bigger biopsy on the surface (atleast 8 mm) and depth (atleast 5 mm), but this does not entirely solve the issue [6,20]. Furthermore, it should be emphasised that the sample should be deep enough to include both the tumour's invasive front and its supporting healthy tissue [5]. It is necessary to include the invasive front with stroma in the biopsy in order to examine the tumour microenvironment and other tumour components. The reliability of the biopsy depends on the depth of the sample [5,6,14].

Limitation(s)

Limitations of the present study are small sample size and lack of follow-up. Furthermore studies on the large sample size are needed.

CONCLUSION(S)

To sum up, different histological markers that are simple to measure on common H&E stained sections can be used to predict the prognosis of OSCC. In early-stage OSCC, tumour budding is a simple and reliable predictive indicators that are also linked to a higher chance of cervical LN metastases, which is linked to a worse outcome. As a result, tumour budding to be routinely assessed in resection and in preoperative biopsy specimens of oral cancers and should be a part of standard reporting format for OSCC. This may aid in the individualisation of treatment for these patients.

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