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CLINICAL & BASIC RESEARCH

A Correlation of Tumour Budding and Tumour-Stroma Ratio with Clinicopathological Factors in Oral Squamous Cell Carcinoma

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abstract: ***Objectives:*** This study aimed to analyse the association of tumour budding (TB) and tumour-stroma ratio (TSR) with clinicopathological parameters that can be easily viewed on routine haematoxylin and eosin (H&E)-stained slides to provide an easy and cost-effective method for prognosticating oral squamous cell carcinoma (OSCC). ***Methods:*** This study was conducted at the ESIC Medical College and Hospital in Faridabad, India, from July 2022 to October 2022. In patients with histologically diagnosed OSCC, TB and TSR were evaluated via routine H&E-stained sections and correlated with clinicopathological parameters. Statistical analysis was performed using Chi-squared test. ***Results:*** A total of 50 patients were included. The mean age of participants was 61 ± 12.72, and the male-to-female ratio was 7.1:1. Most of the tumours were located on the tongue (46%), followed by the buccal mucosa (26%), gingivobuccal sulcus (12%) and retromolar trigone (8%). The palate and alveolus were the other sites involved, constituting 4% each. TB and TSR were both found to be significantly associated with the tumour grade, lymph node metastasis and tumour size. A highly significant correlation was also found between TB and TSR (P = 0.001). ***Conclusions:*** Both TB and TSR can be easily evaluated on routine H&E sections; they are highly reproducible and were found to be reliable independent prognostic markers in OSCC. Therefore, this simple and cost-effective method of prognostication, which is currently lacking in clinical practice, will help clinicians to identify patients with poor prognosis and thus individualise their treatment plan.

Keywords: Tumour microenvironment; Oral Squamous Cell Carcinoma; India.

Advances in Knowledge

- *Tumour budding (TB) and tumour-stroma ratio (TSR) were both found to be significantly associated with the tumour grade, lymph node metastasis and tumour size in oral squamous cell carcinoma (OSCC).*
- *A highly significant correlation was also found between TB and TSR in OSCC.*

Application to Patient Care

- *TB and TSR can be easily performed on routine haematoxylin and eosin sections and thus are cost effective.*
- *TB and TSR can be used for prognostication in patients with OSCC and thus may help in deciding the appropriate mode of treatment, such as chemotherapy.*

Oropharyngeal cancer ranks 6th most prevalent malignancy among all cancers worldwide. India contributes one-third of the total oral carcinoma cases globally, and squamous cell carcinoma (SCC) is the commonest carcinoma in the head and neck region.[1] These carcinomas, like any other solid carcinoma, comprise both carcinomas and stroma.[2] The stroma prevents the spread of the tumour in normal tissues; however, in the tumour tissue, it could lead to tumour progression. Tumour-associated stroma and cancer-associated fibroblasts are implicated in tumour progression. Recently, several studies were conducted to evaluate the role of tumour-stroma ratio (TSR) in oesophageal, breast, colon and cervical cancers, and they found it to be an independent prognostic factor. However, the role of TSR in oral SCC (OSCC) is still not clear.[3]

Tumour budding (TB) signifies a pattern of invasion where tumour cells, either in isolation or in small clusters of up to 5 cells, are seen within the stroma.[4] TB is associated with poor prognosis and aggressive tumour behaviour. TB has been studied in several malignancies, including oesophageal carcinoma, colorectal cancer, breast cancer and pancreatic ductal adenocarcinoma.[5–8] Besides the various histological markers, such as tumour differentiation, tumour thickness, pattern of invasion, perineural invasion and extracapsular spread in lymph nodes, numerous molecular studies have been conducted to identify the prognostic biomarkers in OSCC. However, none of the markers identified has been proven to be of significance for use in routine clinical practice.[4] Thus, there are still lacunae in current knowledge and the need for reliable prognostic biomarkers for oral carcinomas still persists.[4]

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Thus, this study aimed to analyse the association of TSR and TB with clinicopathological parameters that can be easily viewed on routine haematoxylin and eosin (H&E) stained slides to provide an easy and cost-effective method for prognosticating OSCC.

Methods

The study was conducted in the Departments of Pathology and Otorhinolaryngology, ESIC Medical College and Hospital, Faridabad, India, from July 2022 to October 2022. All cases of histologically proven OSCC were included in the study. Patients with a history of chemotherapy or radiotherapy and those who did not give consent were excluded.

H&E-stained slides were used for the assessment of TSR and TB in biopsy-proven cases of OSCC. TB is defined as the presence of small tumour nests composed of <5 tumour cells. For evaluating TB, tumour specimen slides were viewed microscopically under ×10 magnification. Subsequently, tumour buds were counted at the most invasive area in 10 fields under ×400 magnification. TB was analysed in two ways: the total number of buds in 10 high-power fields (HPFs) and the maximum number of buds per field in 10 HPFs. Greater than 10 tumour buds/10 HPFs was defined as high total TB; 4–9 tumour buds/10 HPFs was defined as intermediate TB and <4 tumour buds/10 HPFs was defined as low total TB.

To assess TSR, the most populated tumour areas were selected under ×4 magnification; then, TSR scoring was done under ×10 magnification. A stromal cell ratio of ≤50% was considered to be low (low TSR), while a ratio of >50% was considered to be high stroma ratio (high TSR).

Clinical details, including the patients' age (≤50 years and >50 years) and gender, site of the lesion, size of the lesion (<2 cm, 2–4, >4 cm) and pathological details, such as the tumour grade (well differentiated, moderately differentiated and poorly differentiated) and lymph node involvement, were recorded.

Lymph node involvement, in cases of incisional biopsies of primary OSCC where histological examination was not possible, was assessed using other investigation modalities, including fine needle aspiration cytology (FNAC) of a palpable lymph node (if present) and a positron emission tomography-computed tomography (PET-CT) scan (features highly suggestive of lymph node metastasis on radiology were considered positive).

A Chi-squared test was used to evaluate the association of TSR and TB with clinicopathological parameters. A *P* value of <0.05 was considered statistically significant.

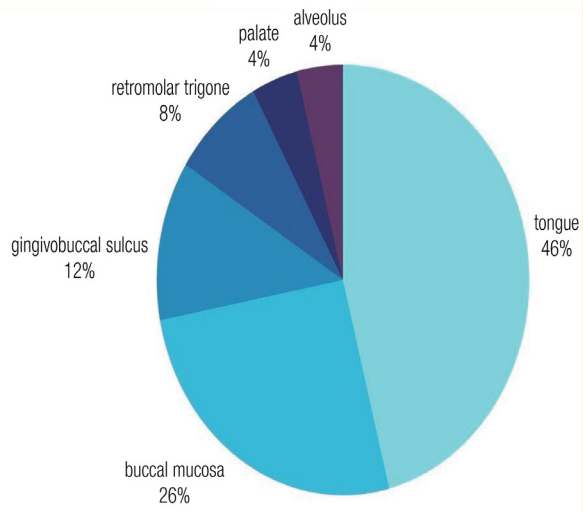

Figure 1: The distribution of oral squamous cell carcinoma at various sites.

Ethical clearance was obtained from the institutional ethical committee (134 X/11/13/2022-IEC/53). Written informed consent was obtained from all the patients prior to the commencement of the study.

Result

A total of 50 patients were included in this study. The mean age was 61 ± 12.72 years and there was a male-to-female ratio of 7.1:1. Most of the tumours were located on the tongue (46%), followed by the buccal mucosa (26%) gingivobuccal sulcus (12%), retromolar trigone (8%) and tonsil (8%). The palate and alveolus were the other sites involved, constituting 4% each. [Figure

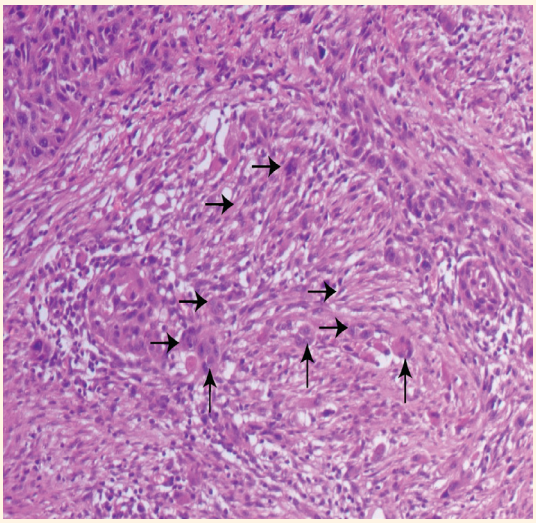

Figure 2: Haematoxylin and eosin stain at ×100 magnification showing high tumour budding (arrows) at the invasive front of oral squamous cell carcinoma.

Table 1: Correlation of tumour budding and tumour-stroma ratio with clinicopathological parameters.

Characteristic	TB				TSR		
	Low (0–4)	Intermediate (4–9)	High (>10)	*P* value*	Low	High	*P* value*
Age in years							
≤50 (n = 23)	12	4	7	0.560	7	16	0.202
>50 (n = 27)	10	6	11		13	14	
Gender							
Male (n = 43)	19	8	16	0.808	18	25	0.505
Female (n = 7)	3	2	2		2	5	
Grade							
1 (well differentiated; n = 13)	10	2	1	**0.0281**	1	12	**0.010**
2 (moderately differentiated; n = 30)	11	7	12		14	16	
3 (poorly differentiated; n = 7)	1	1	5		5	2	
N stage (LN metastasis; n = 21)							
Present	5	6	10	**0.05**	14	7	**0.001**
Absent	17	4	8		6	21	
Size of tumour							
<2 (n = 22)	15	2	5	**0.019**	3	19	**0.003**
2–4 (n = 12)	2	5	5		7	5	
>4 (n = 16)	5	3	8		10	6	

TB = tumour budding; TSR = tumour-stroma ratio; LN = lymph node
**Using Chi square test.*

1]. The maximum number of cases of OSCC (60%) belonged to histological Grade 2, followed by Grades 1 (26%) and 3 (14%). Of the 50 specimens, 11 were modified radical neck dissection (MRND) specimen, while the rest were incisional biopsy specimens.

No significant correlation was found between TSR and TB with age and gender [Table 1]. TB and TSR in OSCC were found to be significantly associated with histological tumour grade (P <0.0281 and 0.010, respectively), where a high TB and low TSR were seen in higher-grade tumours [Table 1; Figures 2 and 3A].

Lymph node metastasis was observed in 21 cases. Of these 21 cases, 11 were MRND specimens, 3 were positive on FNAC of palpable cervical lymph nodes and the rest (n = 7) were considered positive based on radiological findings on PET-CT which were highly suggestive of metastasis. A significant association of TSR with lymph node metastasis was found (P = 0.001). Similarly, a significant association of TB and TSR with tumour size was found (P = 0.019 and 0.003, respectively). A highly significant association was also observed between TSR and TB (P = 0.001) [Table 2]. In addition, those with low TB had a high TSR and vice versa [Figure 3B].

Table 2: A correlation between tumour budding and tumour-stroma ratio.

	TSR high	TSR low	*P* value*
TB low	19	3	0.0001
TB intermediate	7	3	
TB high	4	14	

TSR = tumour-stroma ratio; TB = tumour budding
**Using Chi squared test.*

Discussion

The various histopathological factors currently being used to prognosticate and select the initial treatment, adjuvant therapy and follow-up for OSCC include tumour grade, mode of invasion, pattern of invasion, lymphovascular invasion, perineural invasion, depth of invasion, extracapsular lymph node invasion and resection margin status.[9]

The invasive tumour front has been studied in recent years. The cancer cells at the invasive front, in comparison to those in the superficial or central regions of the tumour mass, are more aggressive.[9]

TB is a process where tumour cells, either singly or in clusters of up to 5 cells, detach from the tumour

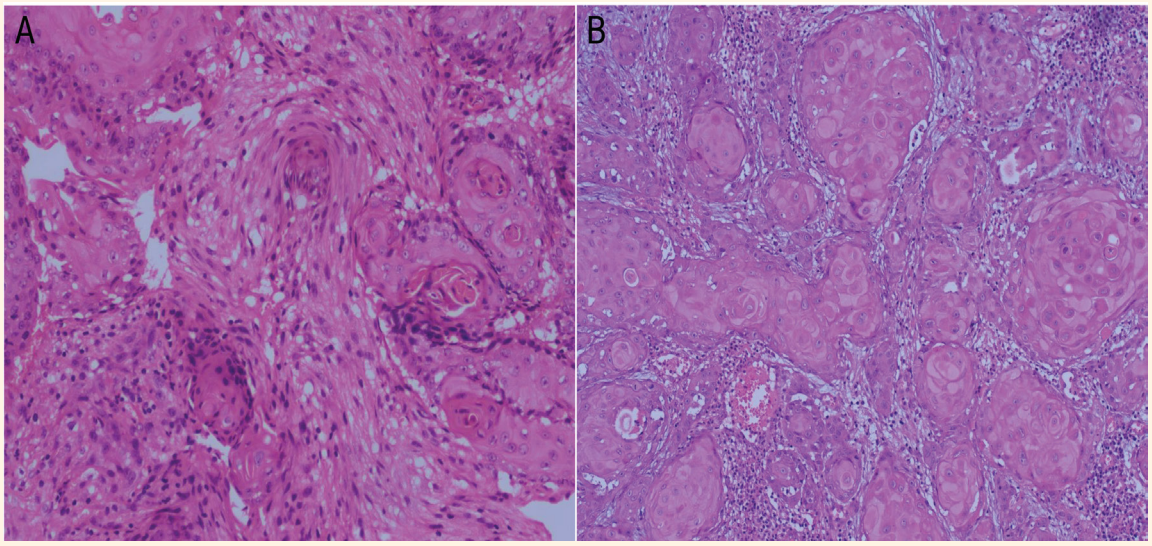

Figure 3: Haematoxylin and eosin stains at ×400 magnification showing **(A)** low tumour-stroma ratio in oral squamous cell carcinoma and **(B)** high tumour-stroma ratio in oral squamous cell carcinoma.

mass and invade the surrounding normal tissue. TB has been studied in colorectal tumours and was found to be a reliable and reproducible prognostic factor in these tumours.[10]

This may be due to TB's association with the nuclear location of b-catenin, which is connected to E-cadherin aberrations. Also, a loss of expression of epithelial cell adhesion molecules is present, which may be responsible for these alterations.

A meta-analysis by Almangush *et al.*, which included 16 studies, evaluated the prognostic significance of TB and found a higher TB to be significantly associated with lymph node metastasis and disease-free and overall survival rates.[12]

In the current study, the authors also found a significant association between TB and lymph node metastasis. Xie *et al.* found a correlation between TB and occult lymph node metastasis in early-stage OSCC, which is the most common reason for relapse and poor prognosis in the early stages.[13] A study by Angadi *et al.*, which included 75 cases of OSCC, found high-intensity TB to be an independent prognostic factor of lymph node metastasis, similar to the current study's finding.[14] However, they found no significant association between TB and patient age or gender or tumour site, size, grade or stage. In addition, the authors found that advanced tumour grade and size were significantly associated with higher TB, which is in accordance with Jensen *et al.* and Zhang *et al.'s* findings.[11,15] Zhang *et al.* also found high-grade TB to be associated with higher T stage, smaller nest size, larger nuclear diameter, advanced clinical stage, poorly pathological differentiation and higher TSR.[11]

In the current study, majority of the samples were incisional biopsies (n = 39). Few studies have evaluated the prognostic value of TB in biopsy specimens of OSCC.[16,17]

The small sample size of the current study, the lack of an infiltrative front, fragmentation, artifacts and extensive necrosis may have prevented an accurate assessment of the biopsy. However, preoperative assessment of TB may be helpful in determining the prognosis, where TB is correlated with grading, depth of invasion and lymph vascular invasion.[18] Therefore, for proper evaluation, it is recommended that the biopsy include clinically healthy tissue with a horizontal margin of ≥8 mm and a vertical margin of ≥5 mm or several incisional biopsies be performed.[19] In several epithelial cancers, TSR has been found to be an independent prognostic factor. TSR is a simple, reliable and inexpensive procedure since it can be easily evaluated on H&E-stained slides, and thus, TSR scoring can be a part of routine histopathological report. Zhang *et al.* found that patients with laryngeal SCC who had higher TSR showed worse prognosis.[11] The current study found a significant association of TSR with the tumour size and lymph node metastasis, where cases with a lower TSR had higher tumour sizes and risks of lymph node metastasis.

A review of solid tumours by Wu *et al.* suggested that a higher proportion of stroma is associated with adverse features such as advanced depth of invasion, tumour aggressiveness in the form of advanced clinical stage and positive lymph node metastasis.[19] The adverse prognosis of patients with tumours which have a higher proportion of stroma may be due to the interactions between tumour cells and cancer-associated fibroblasts (CAF). The role of CAFs in the progression and metastasis of OSCC have been reported.[10]

Another review of studies on head and neck cancers by Almangush *et al.* suggested a significant correlation of TSR with features of aggressive tumour behaviour, such as perineural invasion, depth of infiltration, cell-in-cell invasion, advanced stage and treatment resistance.[2] Rani *et al.* found a significant correlation between TSR and tumour size as well as advanced tumour stage.[20] However, Mascitti *et al.* found no significant association between TSR and clinicopathological parameters.[21] Similarly, Ünlü *et al.* also found no association between TSR and clinical parameters such as tumour location, histological grade, clinical stage and perinodal invasion.[22] In the current study, low TSR was found to be significantly associated with higher histological tumour grade, larger tumour size and positive lymph node metastasis. Also, Ablahad *et al.* reported no significant correlation between TSR and patient age and gender as well as tumour site and grade in cases of OSCC.[23] The current study found no significant association between TSR and patient age and gender. However, a highly significant correlation was found between TSR and TB, where a higher TB was associated with a higher stroma proportion, i.e. a lower TSR.

The limitation of the current study is its small sample size. Therefore, more multi-institutional studies with larger sample sizes are required.

Conclusion

TB and TSR can both be used to evaluate the prognosis of patients with OSCC. In the current study, TB and TSR showed a significant association with lymph node metastasis and tumour size. TB was also found to be significantly associated with tumour grade. However, no significant correlation was found between TB and TSR and parameters such as patient age and gender. Both TB and TSR can be easily evaluated on routine H&E sections, and they have been found to be highly reproducible and reliable independent prognostic markers in OSCC. Thus, this simple and cost-effective method of prognostication, which is currently lacking in clinical practice, will help clinicians to identify patients with poor prognosis and thus individualise their treatment plan.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTION

KS conceptualised and designed the study. LV conducted the literature review. LV and AJ collected the data. LV and KS drafted the manuscript. MP, MJ, RKC, CA, VC, SR and AJ edited the manuscript, and all authors critically reviewed the manuscript. All authors approved the final version of the manuscript.

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