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

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Abstract

Background: Squamous cell carcinoma is the most common carcinoma in the head and neck region. Both tumor budding and tumor stroma ratio are being studied in the recent years in various solid tumors for their role as a prognostic marker, however the studies in oral squamous cell carcinoma are limited. ***Methods:*** A total of 50 patients of oral squamous cell carcinoma proved histologically were included in the study over a period of 4 months (July 2022-October 2022). Tumor budding(TB) and Tumor stroma ratio (TSR) were evaluated on routine hematoxylin and eosin stained sections and these were correlated with clinicopathological parameters. Statistical analysis was done using Chi-square test and p value <.05 considered significant. ***Result:*** The mean age was 52.72 +_ 13.16 and M: F of 7.1:1. Most of the tumors were located on tongue (46%) followed by buccal mucosa (26%), gingivobuccal sulcus (12%) and retromolar trigone (8%). Palate and alveolus were the other sites involved constituting 4% each. Both TB and TSR were found to be significantly associated with grade of the tuumor, lymph node metastasis and size of the tumor. A highly significant correlation was also found between Tb and TSR with a p value <.001. ***Conclusion:*** Both TB and TSR can be easily evaluated on routine H&E sections and are highly reproducible and found to be reliable independent prognostic markers in OSCC. Thus, this simple and cost-effective method of prognostification which is currently lacking will help in identifying patients with poor prognosis and thus, individualise the treatment plan.

Keywords: Tumor Budding; Oral squamous cell carcinoma; Tumor stroma ratio.

Advances in Knowledge

1. Tumor Budding and Tumor stroma ratio are a topic of recent interest and it is being studied in a number of tumors.
2. Our study aims to provide an insight about Tumor budding and tumor stroma ratio in Oral squamous cell carcinoma.

Application to patient care

1. Both Tumor budding and Tumor stroma ratio are recently being used as an additional prognostic marker in many tumors.
2. Tumor budding and Tumor stroma ratio can be easily performed on routine H&E sections and thus cost effective.
3. Tumor budding and Tumor stroma ratio can be used for prognostication of patients with oral squamous cell carccinoma and thus may also help in deciding the treatment including chemotherapy.

Introduction

The cancer of the oral cavity and the pharynx ranks sixth worldwide. India contributes to one-third of the total oral carcinoma ceases globally.[1] Squamous cell carcinoma (SCC) is the most common carcinoma in the head and neck region. These carcinomas consist of both carcinomas and stroma like other solid carcinomas.[2] The stroma prevents the spread of tumor in normal tissues, however, in the tumor tissue it could lead to tumor progression. The tumor associated stroma and cancer associated fibroblasts are implicated in the tumor progression phases. Recently, several studies are done for the evaluation of tumor stroma ratio(TSR) in Esophageal cancer, Breast cancer, Colon cancer, and Cervical cancer and found to be an independent prognostic factor. However, the role of TSR in oral squamous cell carcinoma is still not clear.[3]

Tumor budding signifies a pattern of invasion where either isolated tumor cells or tumor cells in small clusters (up to 5 cells) are seen within the stroma. Tumor budding is associated with poor prognosis and aggressive behaviour of the tumor.[4]Tumor budding has been studied in several malignancies including ,esophageal carcinoma,[5] colorectal cancer,[6] breast cancer[7] and pancreatic ductal adenocarcinoma.[8] Besides the various histological markers, like tumor differentiation, thickness of tumor, pattern of invasion, perineural invasion, extracapsular spread in lymph nodes, numerous molecular studies are done to identify the prognostic biomarkers in Oral SCC. But none of them has been

proven to be of signifcance to be used in routine practice.[4] Thus, there is still lacunae in our knowledge and the need for reliable prognostic markers for oral carcinomas still needs to be addressed.[4]

Thus in this study we aim to analyse the association of TSR and TB with the clinicopathological parameters which can be easily done on routine Hematoxylin and eosin stained slides, providng an easy and cost effective method for prognostication of Oral SCC.

Methods

The study was conducted in the Department of Pathology and Department of Otorhinolaryngology, ESIC Medical College and Hospital, Faridabad within a period of four months (July 2022-October 2022).

Inclusion criteria - All the cases of histologically proven oral squamous cell carcinoma in a period of 4 months were included in the study (July 2022-October 2022). Exclusion criteria -. The patients with a history of chemotherapy or radiotherapy and all the patients who did not give consent were excluded from the study.

Hematoxylin and eosin stained slides were used for the assessment of TSR and TB in biopsy proven cases of OSCC. Tumor budding is small tumor nests composed of <5 tumor cells. For evaluating tumor budding, tumor slides were scanned at 10× objective. Subsequently, tumor budding was counted at the most invasive area in 10 fields at ×400 magnification. The tumor budding was analyzed by two ways: the total numbers of tumor budding under 10 HPFs and the maximum numbers per field among 10 HPFs. >10 tumor budding/10 HPFs was defined as high total tumor budding. 4-9/10 HPF as intermediate and <4 tumor budding/10 HPFs as low total tumor budding.

For TSR assessment, the most tumor areas were selected with 4× objective, then, TSR scoring were evaluated using 10× objective. Stromal cells ratio ≤50% were taken as low stroma ratio (low TSR) and >50% as high stroma ratio (high TSR).

The clinical details including the age (<50 years and >50 years) and gender of the patient, site of the lesion , size of the lesion (<2 cm, 2-4, >4 cm) along with pathological details like grade of the tumor (well differentiated, moderately differentiated and poorly differentiated) and lymph node involvement was recorded.

Lymph node involvement in cases of incisional biopsies of primary oral SCC where histological examination was not possible was assessed by using other investigation modalities including fine needle aspiration cytology (FNAC) from palpable lymph node if present, and radiological assessment through PET CT and features highly suggestive of lymph node metastasis on radiology was considered positive.

Ethical clearance was taken from the Institutional ethical committee. A written informed consent was taken from the patients.

Statistical analysis

Chi-squared method was used for evaluation of association of TSR and TB with clinicopathological parameters.

Result

A total of 50 patients were include in the study with mean age 61±12.72 and M: F of 7.1:1. Majority of Most of the tumors were located on tongue (46%) followed by buccal mucosa (28%), gingivobuccal sulcus(10%), retromolar trigone (8%) and tonsil (8%). Palate and alveolus were the other sites involved constituting 4% each.(46%) followed by larynx (26%) then buccal mucosa (16%) and (4%) in palate, tonsil and alveolus each (Figure 1).The maximum number of cases belonged to histological grade 2 (60%), followed by grade 1 (26%) and grade 3 (14%). Out of the total 50 cases 11 cases were modified radical neck dissection (MRND) specimen and the rest of the cases were incisional biopsies.

We found no significant correlation between TSR & TB with age and gender. (table 1). TB and TSR in OSCC was found to be significantly associated with histological grade of tumor with p value<0.5 (table 1) where higher TB (fig 2) and low TSR (fig 3 A) was seen in higher grade of tumor.

Metastasis to lymph node was found in 21 cases with significant p value of (<0.05). Out of these 21cases, 11 cases were MRND specimen , 3 cases were positive on FNAC from palpable cervical lymph nodes and the rest (7) were considered positive based on radiological findings on PET-CT highly suggestive of metastasis. A significant association of TB & TSR was found with lymph node metastasis. Similarly, the association of TB & TSR with size of tumor significantly found with p value (<0.5). A highly significant association was present between TSR &TB(P=0.001) (table 2). We found that those with low tumor budding have high TSR (fig 3B), and vice versa.

Discussion

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

In the recent years the invasive tumor front is being studied. The cancer cells at the invasive front, in comparison to the cells present in the superficial or central regions of the tumor mass are more aggressive.[9]

Tumor Budding (TB) is process where the tumor cells either singly or clusters of upto five tumor cells detach from the tumor mass and invade the surrounding normal tissue. It has been studied in colorectal tumor and has been found to be a reliable and reproducible prognostic factor in these tumors.[10]

TB may be due to its association with the nuclear location of b-catenin which in turn is connected to E-cadherin aberrations. Also, a loss of expression of epithelial cell adhesion molecule is present. The loss of intercellular adhesions are responsible for these alterations. [11]

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

In the current study also we found a significant association of TB with lymph node metastasis. Xie et al found a correlation between tumor budding and occult lymph node metastasis in early stage OSCC which is the most common reason for relapse and poor prognosis in early stages.[12] A study by Angadi et al included 75 cases of OSCC and found high intensity tumor budding to be an independent prognostic factor of lymph node metastasis similar to our study.[13] However, they found no significant association of TB with age, gender, site, size, grade and stage of the tumor. In addition to that we also found that advanced tumor grade and size of tumor was also found to be significantly associated with higher TB which was in accordance with a study by Jensen et al [14] and Zhang et al.[11] Zhang et al. also found that high-grade tumor budding was associated with higher T stage, smaller nest size, larger nuclear diameter, advanced clinical stage, worse poorly pathological differentiation and higher TSR.[11] The meta-analysis of cases of oral tongue cancer suggested high TB index had a poorer overall survival.[17]

In our study the majority of samples were incisional biopsies (40/50). Few studies had evaluated the prognostic value of TB in biopsy specimens of OSCC.[15,16]

The accurate assessment of the biopsy may be limited by the small sample size, lack of the infiltrative front, fragmentation, artifacts, and extensive necrosis. However, preoperative assessment of TB may be helpful for determining the prognosis where TB was correlated with grading, depth of invasion, lymph vascular invasion.[17] Therefore, for proper evaluation, it is suggested that the biopsy should include clinically healthy tissue with a horizontal margin of $p \geq 8$ mm and a vertical margin of ≥5 mm or perform several incisional biopsies.[17] In several epithelial cancers TSR has been found to be an independent prognostic factor. TSR is a simple, reliable and inexpensive procedure as 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 the patients with higher TSR showed a worse prognosis in laryngeal SCC.[13] We found a significant association of TSR with the size of the tumor and lymph node metastasis where cases with lower TSR had higher tumor size and risk of lymph node metastasis.

The review by Wu et al in solid tumors suggested that the higher proportion of stroma was associated with adverse features like advanced depth of invasion, tumor aggressiveness in the form of advanced clinical stage and positive lymph node metastasis.[18]

The adverse prognosis in patients with tumors having a higher proportion of stroma may be due to the interactions between tumor cells and cancer-associated fibroblasts (CAF). The role of CAFs in the progression of OSCC and metastasis have been reported.[10]

Another review by Almangush et al on the studies involving head and neck cancers suggested a significant correlation with features of aggressive tumor behavior like perineural invasion, depth of infiltration, cell-in-cell invasion, advanced stage and treatment resistance. [19] Rani et al found a significant correlation between TSR and size of the tumor and advanced stage. [20]

However, Masciti et al found no significant association of TSR and clinicopathological parameters. [21] Similarly, Ünlü et al. also found no association of TSR with the clinical parameters like tumor location, histological grade, clinical stage, or perinodal invasion. [22] In the current study we found low TSR was

significantly associated with higher histological grade and also larger size of the tumor and positive lymph node matastasis.

Also, Ablahad et. al reported no significant correlation of TSR with the age, gender, site and grade of the tumor in cases of oral squamous cell carcinoma.[23] We found no significant association of TSR with age and gender in the current study.

We found a highly significant correlation between TSR and TB where higher TB was associated with higher stroma i.e. lower TSR.

The limitations of the current study is smaller sample size, therefore, more multi-institutional studies with a larger sample size are required.

Conclusion

Both TB and TSR can 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 size of the tumor. TB was also found to be significantly associated with the grade of the tumor. However, no significance was found between TB and TSR with the parameters like age and gender of the patients. Both TB and TSR can be easily evaluated on routine H&E sections and they are highly reproducible and found to be reliable independent prognostic markers in OSCC. Thus, this simple and cost-effective method of prognostification which is currently lacking will help in identifying patients with poor prognosis and thus, individualise the treatment plan.

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Conflicts of interest

The authors declare no conflicts of interest.

Authors' Contribution

KS conceptualized 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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Table 1: Correlation of TB and TSR with clinicopathological parameters.

(TB- tumor budding, TSR- Tumor stroma ratio)

		TB	**P (chi square)**			**TSR**		**P (chi square)**
		Low (0-4)	Intermediate (4-9)	High (>10)		Low	High	
Age	<50 (23)	12	4	7	0.560	7	16	0.202
	>50 (27)	10	6	11		13	14	
Gender	Male	19	8	16	0.808	18	25	0.505
	Female (7)	3	2	2		2	5	
Grade	1 (Well differentiated) (13)	10	2	1	**0.0281**	1	12	**0.010**
	2 (Moderately differentiated) (30)	11	7	12		14	16	
	3 (Poorly differentiated) (7)	1	1	5		5	2	

N Stage (LN Metastasis) (21)	Present	5	6	10	**0.05**	14	7	**0.001**
	Absent	17	4	8		6	21	
Size of tumor	<2 (22)	15	2	5	**0.019**	3	19	**0.003**
	2-4 (12)	2	5	5		7	5	
	>4 (16)	5	3	8		10	6	

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296 Table 2: A correlation between TB and TSR

	Tsr high	Tsr low	P value (chi square)
TB low	19	3	**0.0001**
TB intermediate	7	3	
TB high	4	14	

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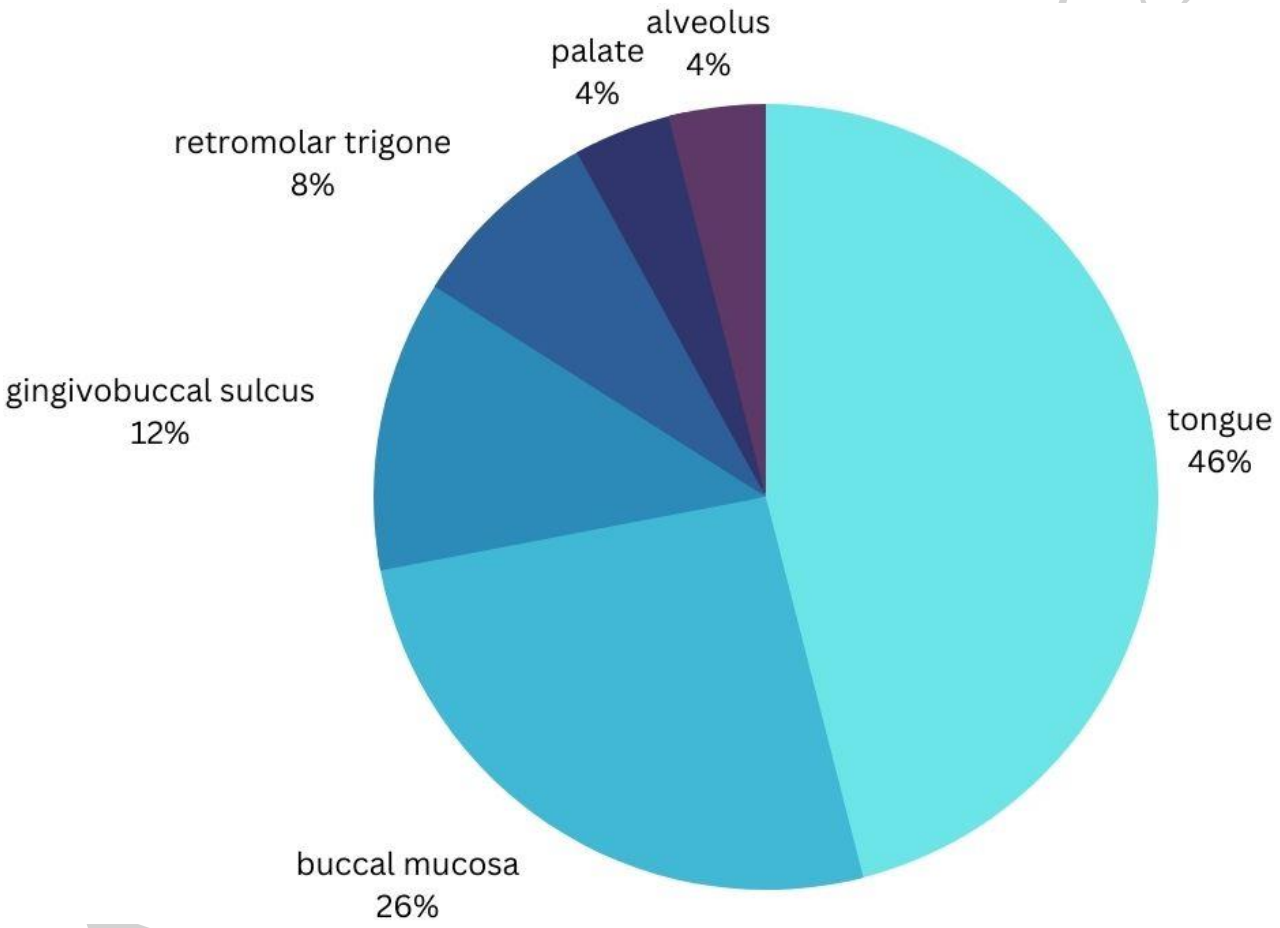

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300 Figure 1: Distribution of OSCC at various sites.

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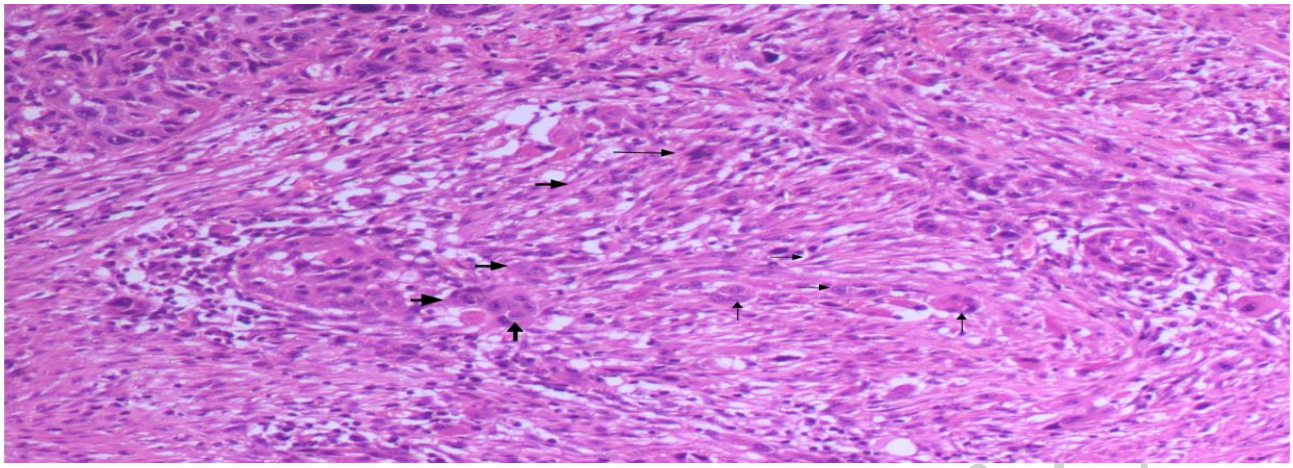

Figure 2: H&E stained sections show high Tumor budding (TB) at the invasive front of OSCC.(100X)

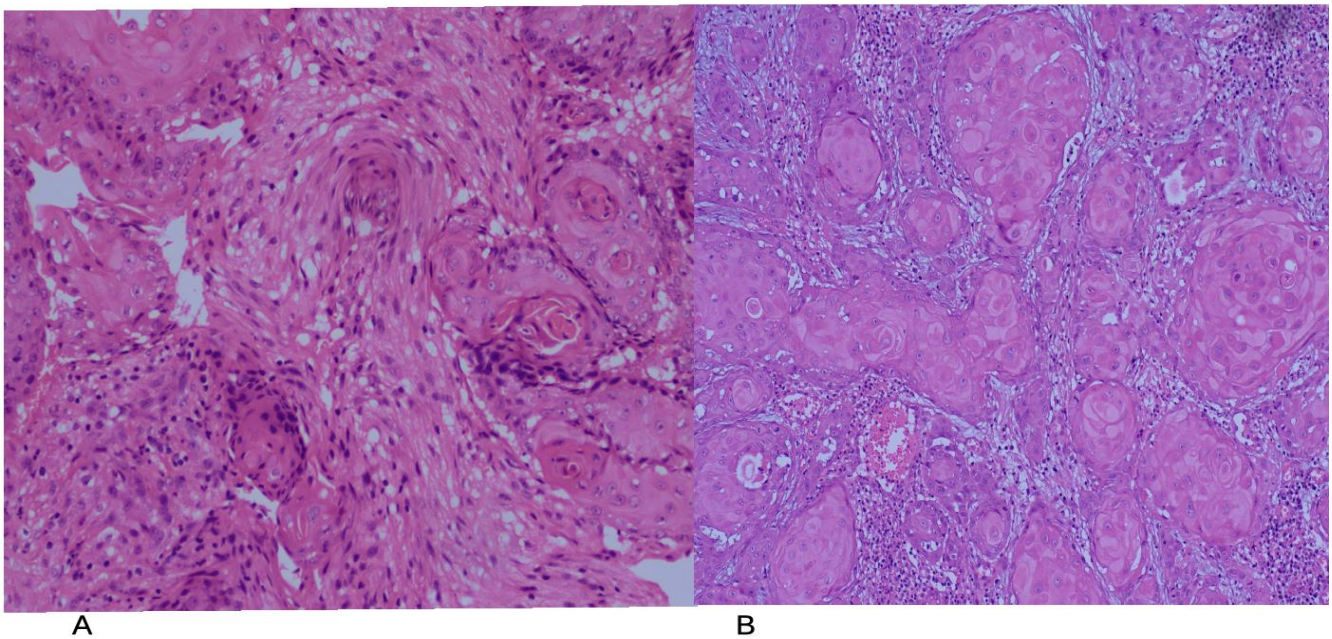

Figure 3: A) H&E stained sections shows low Tumor stroma ratio (TSR) in OSCC.(400X). B) H&E stained sections show high Tumor stroma ratio (TSR) in OSCC.(400X)