Prognostic and Predictive Factors in Gingivo Buccal Complex Squamous Cell Carcinoma: Role of Tumor Budding and Pattern of Invasion

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Abstract Invasive tumor front (ITF) is the deepest three to six cell layers or detached tumor cell groups at the advancing edge of the tumor. Tumor budding is defined as presence of isolated single cells or small cell clusters scattered in the stroma ahead of the ITF and is characteristic of aggressive cancer. It is recognized as an adverse prognostic factor in several human cancers like colorectal, oesophageal, laryngeal cancers and more recently tongue cancers. However, the prognostic value of tumor budding has not been reported in GBCSCC. The aim of our study was to evaluate the role of pattern of invasion (POI) at the ITF, Tumor budding and other clinicopathological parameters in predicting nodal metastases and prognosis in GBCSCC. 33 patients with primary GBCSCC were prospectively evaluated at a tertiary care referral centre. Tumor budding and type of POI was examined in detail and data documented. Statistical analyses were carried out to assess the correlation of tumor budding, POI, and other clinicopathologic parameters (stage, grade of the tumor,

tumor thickness, PNI, LVI) with nodal metastases and predict prognosis. Cox regression was used for both Univariate and multivariate analysis. Significant predictors of nodal metastases on Univariate analysis were male gender ($p = 0.021$), smoking ($p = 0.046$), Tumor budding ($p = 0.014$) and diffuse infiltrative/worst POI ($p = 0.004$), where as on multivariate analysis only worst POI was significantly associated with positive lymph nodes ($p = 0.004$). Presence of nodal metastases ($p = 0.01$) and tumor thickness >5 mm ($p = 0.009$) were independent negative prognostic factors on multivariate analysis. Significant single risk factor predictive of positive lymph nodes is worst POI in GBCSCC. Nodal metastases and >5 mm tumor thickness are independent risk factors for disease free survival.

Keywords Tumor budding · Pattern of invasion · Gingivo buccal squamous cell carcinoma · Prognostic factor

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Introduction

Gingivo buccal complex, which includes buccal mucosa, gingivo-buccal sulci and upper and lower alveolus are the most common subsites of oral cancer in India. Nearly two thirds of all oral cancers are located in the gingivo buccal complex (GBC). Though oral cavity is easily accessible for visual examination, about 60 % of patients present only at an advanced stage [1]. Most of these patients have nodal metastases at the time of presentation. The reported occult metastases rate is about 26 % in all stages of buccal cancer [2]. At present therapeutic decision making is based on tumor, node, metastasis (TNM) Staging supplemented by various clinicopathological prognostic and predictive

factors. Lymph nodal status is the most significant adverse prognostic factor and a major determinant of poor survival. Several studies have shown tumor thickness to correlate with survival. Improved survival is noted when tumor thickness is less than 6 mm in gingivo buccal complex squamous cell carcinoma (GBCSCC) [3]. Despite significant development in multimodality management of GBCSCC locoregional recurrence occurs. At present more than half the patients fail therapy indicating that more aggressive treatment or individualized therapy is warranted for this group. Despite major efforts to identify new predictive parameters and histological systems, clinical features are still the most reliable prognostic factors for patients with oral SCC. Hence there is a growing need for new prognostic strategies and biomarkers that will help in clinical decision making and management of patients.

Invasive tumor front (ITF) is defined as the deepest three to six cell layers or detached tumor cell groups at the advancing edge of the tumor. Cancer cells located in the ITF have been suggested to be more aggressive in terms of metastatic potential and influence prognosis [4]. Pattern of invasion (POI) refers to the manner in which tumor infiltrates host tissue at the tumor/host interface. Cancers infiltrating in a widely dispersed manner are thought to be more aggressive than those infiltrating in a broad pushing fashion. Bryne et al. had developed a multiparameter histopathological grading system based on pattern of tumor invasion (POI), degree of keratinisation, nuclear pleomorphism and host response for prognosis of oral cancer [5, 6]. Similar histologic risk scoring scheme for decision making and adjuvant treatment in head and neck cancer has been developed and validated by Brandwein-Gensler [7, 8]. Although several such histopathological parameters and grading systems have been developed and refined in literature, none is universally accepted.

Tumor budding is another histopathological parameter, that has been recognized as an adverse prognostic factor in several human cancers like colorectal, oesophageal, laryngeal cancers and more recently tongue cancers [9–14]. It is defined as presence of isolated single cells or small cell clusters scattered in the stroma ahead of the ITF and is characteristic of aggressive cancer. It can be easily examined on routine histopathological examination with reproducible results [9]. However, to our knowledge evaluation of tumor budding and its significance in GBCSCC has not been reported.

Therefore we selected all those previously suggested clinicopathological parameters with important prognostic relevance in GBSCC and in addition included tumor budding and POI that are easy and practical to evaluate. The aim of our study was to evaluate the role of POI, Tumor budding and all other clinicopathological parameters in predicting nodal metastases and prognosis in GBCSCC. All

the parameters included in this study are defined in the next section.

Materials and Methods

Patient Cohort

Clinicopathological features of 33 patients (9 male, 24 female), age range (26–84 years) with primary GBCSCC from the Department of ENT and Head and Neck Surgery, Bangalore Baptist Hospital from January 2012 to January 2014 were prospectively analyzed. Tumor staging was according to TNM staging by UICC. None of the patients in the study group had received any form of adjuvant therapy prior to surgery. All 33 patients underwent curative intent resection of primary tumor with unilateral neck dissection. Regular 2 monthly clinical follow up was carried out in all patients. Locoregional recurrences and distant metastases were identified during follow up and managed accordingly.

Histopathological Evaluation

Sections from paraffin blocks were taken such that the slides included the deepest portion in terms of tumor host interface or invasive front. Tumor thickness or depth was measured from the surface of the mucosa to the deepest portion of the tumor. Although various cutoff points have been suggested in different studies in different subsites, depth of invasion of 5 mm seems to be common, and hence 5 mm is the cut off taken in our study. Lymphovascular invasion and perineural spread was also recorded. Bryne et al. had developed a multiparameter 4 point histopathological grading system based on pattern of tumor invasion (POI), degree of keratinisation, nuclear pleomorphism and host response for prognosis of oral cancer [5, 6]. This method has been used in many later studies to evaluate prognosis. However it is cumbersome and reproducibility is doubted. In our study, Pathologist who was blind to all clinical data divided POI findings into 2 groups for ease of interpretation, analysis and reporting. Group 1 included pushing, well delineated broad infiltrating fronds/strands (Bryne 1,2 groups [5, 6]); Fig. 1, and Group 2 (Diffuse infiltrative front/ Worst POI); included strands of infiltrating tumor cells, widespread cellular dissociation into tumor islands or individual tumor cells (Bryne 3,4 groups [5, 6]); Fig. 2a, b.

Scattered foci of isolated single cells or clusters composed of less than 5 cancer cells seen in the stroma ahead of the ITF was noted as tumor budding [9]. After selecting a field in which maximum budding intensity was observed the number of foci was counted. Using 10 foci as cutoff value [12] tumor budding was divided into two groups; group 1 included low

Fig. 1 Broad/pushing pattern of invasion at the invasive tumor front (Group 1) on low power (10×)

intensity budding (0–10 foci) and group 2 included high intensity budding (>10 foci); Fig. 2c.

Statistical Analysis

The predictive significance of clinico-pathological factors in nodal metastasis was assessed using Fisher's exact test, univariate and multivariate logistic regression analyses. The prognostic significance of clinicopathological factors on disease-free survival was assessed using Cox's multivariate proportional hazards regression analysis. The results were considered significant when $p < 0.05$. The end point for Cox's proportional hazards regression analysis of disease-free survival was time to first disease failure/death versus duration of failure free follow up.

Results

The distribution of demographic and clinicopathological variables in all 33 patients is shown in Table 1. Broad or pushing POI was seen in 9 (27.3 %) patients where as Worst POI was seen in 24 (72.7 %) patients. Low intensity tumor budding (0–10 foci) was seen in 12 (36.5 %) patients where as high intensity tumor budding was seen in 21 (63.6 %) patients. Three patients expired because of local recurrence and distant metastasis. Six patients had loco-regional recurrence and are alive on supportive care, whereas 24 patients are alive without disease at the end of the follow-up period. The mean follow-up period was 15 months (range, 6–30 months).

Significant predictors of nodal metastases on Univariate analysis (Table 2) were male gender ($p = 0.021$), smoking

Table 1 Frequency distribution of clinical and histopathological variables

Variable	N	%
Sex		
Male	9	27.3
Female	24	72.7
Alcohol consumption		
Yes	7	21.2
No	26	78.8
Chewing tobacco		
Yes	30	90.9
No	3	9.1
Smoking		
Yes	8	24.2
No	25	75.8
Stage		
T1	5	15.2
T2	14	42.4
T3	3	9.1
T4	11	33.3
Differentiation		
Poor	2	6.1
Moderate	29	87.9
Well	2	6.1
Lymph nodal status		
Positive	18	54.5
Negative	15	45.5
Extracapsular Spread		
Present	6	18.2
Absent	27	81.8
Perineural invasion		
Present	7	21.2
Absent	26	78.8
Lymphovascular invasion		
Present	6	18.2
Absent	27	81.8
Status		
Recurrent disease/mortality	7	21.2
Alive without disease	26	78.8
Pattern of invasion		
Pushing/broad front	9	27.3
Diffuse infiltrative front/worst POI	24	72.7
Tumor budding		
0–10 foci (low intensity)	12	36.4
>10 foci (high intensity)	21	63.6

($p = 0.046$), Tumor budding ($p = 0.014$) and diffuse infiltrative/worst POI ($p = 0.004$), where as on multivariate analysis only worst POI was significantly associated with positive lymph nodes ($p = 0.004$). Presence of nodal

Table 2 Correlation of clinicopathological variables with Lymph nodal metastases

	Nodes—positive n = 18	Nodes—negative n = 15	Unadjusted odds ratio	p value	Adjusted odds ratio
Gender					
Male	8(88.9)	1(11.1)	11.2	0.021	11.5
Female	10(58.3)	14(41.7)	1.202–104.329		0.82–159.8
Smoking					
Yes	7(87.5)	1(12.5)	8.9	0.046	
No	11(44.0)	14(56.0)	0.95–83.616		
Tumor budding					
0–10 foci (low intensity)	3(16.7)	9(60.0)	7.5	0.014	
>10 foci (high intensity)	15(83.3)	6(40.0)	1.49–37.66		
Differentiation					
Well	2(100.0)	0(0)	–	0.271	
Moderate	12(41.4)	17(58.6)			
Poor	1(50)	1(50.0)			
Perineural invasion					
Present	6(85.7)	1(14.3)	0.14	0.095	
Absent	12(46.2)	14(53.8)	0.02–1.36		
Pattern of invasion					
Pushing/broad front	1(11.1)	8(88.9)	19.43	0.004	19.8
Diffuse infiltrative front/ worst POI	17(70.8)	7(29.2)	2.032–185.724		1.6–245.5
Tumor thickness					
<5 mm	2(33.3)	4(66.7)	0.34	0.375	
>5 mm	16(59.3)	11(40.7)	0.05–2.22		
Lymphovascular invasion					
Present	4(66.7)	2(33.3)	1.85	0.665	
Absent	14(51.9)	13(48.1)	0.29–11.9		
Bone involvement					
Present	5(50)	5(50)	0.89	0.88	
Absent	9(52.9)	8(47.1)	0.186–4.24		
Skin involvement					
Present	2(100)	0	–	1.00	
Absent	8(80)	2(20)			
T stage					
T1	3(60.0)	2(40.0)	1.25	0.89	
T2	8(57.1)	6(42.9)	0.15–10.69		
T3	1(33.3)	2(66.7)	1.11		
T4	6(54.5)	5(45.5)	0.23–5.45		
			0.42		
			0.03–6.06		

metastases ($p = 0.01$) and tumor thickness >5 mm ($p = 0.009$) were independent negative prognostic factors on both univariate and multivariate analysis (Tables 3 and 4).

Discussion

Tumor differentiation, pattern of invasion, mitotic activity, nuclear polymorphism, microvascular invasion, lympho-

plasmacytic infiltration and histologic grade of the malignancy have all been reported to affect overall survival [15–17]. However, these factors reflect the characteristics of the entire tumor, and have only a slightly significant effect on prognosis. Generally, most tumors consist of heterogeneous cell populations with variable biologic behavior, and tumor behavior is dependent on a complex interrelationship between tumor and host. Accumulating evidence now suggests that characteristics of the ITF provide most useful

Table 3 Univariate analysis for disease free survival

	Unadjusted hazard ratio	95 % Confidence interval	P value
Nodes	3.55	1.36–9.25	0.01
Positive			
Negative			
Tumor thickness	7.4	1.7–33.0	0.009
<5 mm			
>5 mm			
Smoking	0.78	0.28–2.14	0.64
Sex	0.76	0.29–1.97	0.57
Age	1.01	0.98–1.05	0.43
Differentiation	0.92	0.31–2.77	0.88
Tumor budding	1.32	0.59–2.95	0.49
Pattern of invasion	1.39	0.59–3.29	0.45
Perineural invasion	0.89	0.34–2.27	0.81
T stage	0.87		
	0.64	0.19–6.95	1.16
	0.99	0.11–3.94	0.65
Bone involvement	2.44	0.41–14.64	0.33
Skin involvement	–	–	–

Univariate analysis showing that Positive nodes and Tumor thickness > 5mm are significant for reduced disease free survival

Table 4 Adjusted (Multivariate) Cox's proportional hazards regression analysis of disease-free survival

	Adjusted hazard ratio	95 % C.I.	P value
Nodes	4.03	1.40–11.6	0.01
Positive			
Negative			
Tumor thickness	8.2	1.78–37.8	0.009
<5 mm			
>5 mm			

Adjusted (Multivariate) Cox's proportional hazards regression analysis showing that Positive nodes and Tumor thickness > 5mm confirm reduced disease-free survival

prognostic information [5]. Earlier models by Bryne et al. [5, 6], Bundgaard et al. [18] involved multiparameter histopathological grading system based on pattern of tumor invasion (POI), degree of keratinisation, nuclear pleomorphism and lymphocytic host response for prognosis of oral cancer. Similarly later a novel histologic risk scoring scheme was developed and validated by Brandwein-Gensler [7, 8] which included perineural invasion in addition to Bryne's parameters for decision making and adjuvant treatment in head and neck cancer. Although several such histopathological parameters, grading systems and risk models have been described in literature, none is universally accepted because their clinical utility and reproducibility have not been demonstrated. Often there

has been lack of agreement between observers which greatly reduces the validity of these systems, suggesting that it may need fine-tuning, perhaps by clarification of definitions, simplification of categories or omission of less reproducible variables.

Role of POI in GBCSCC

In our study, our intention was to simplify and build upon the previous studies regarding the predictive value of POI at the tumor host interface. We divided POI into 2 groups. In accordance with previous studies [5, 7, 18, 19] we found that Group 2 (diffuse infiltrative front or Worst POI) had statistically significant association with positive lymph nodes on both univariate and multivariate analysis. Tumors that invaded in small groups, strands or with widespread cellular dissociation showed a higher tendency to metastasize to regional lymph nodes ($p = 0.004$) compared with tumors that invaded in pushing fronts or broad cords (Table 2). This association of worst POI with positive lymph nodes in turn impacts upon survival, as it is well established that nodal status is the single most significant adverse prognostic factor in Oral SCC. POI of the tumour may help in identifying patients at higher risk of having lymph node metastasis and seems to be important in treatment planning (Figs. 2, 3).

Role of Tumor Budding in GBCSCC

Unlike ITF grading and POI, Tumor budding has been shown to have good reproducibility. Another advantage is it can be easily adapted to routine hematoxylin-eosin (H&E) staining based histopathological examination without the need for additional expensive techniques. High intensity Tumor budding has been recognized as an adverse prognostic factor in several human cancers like colorectal, oesophageal, laryngeal cancers and more recently tongue cancers [9–13]. However, little is known about the role of Tumor budding in GBCSCC. In our study, we found that high intensity tumour budding was associated with positive lymph nodes (Table 2) only on univariate analysis ($p = 0.014$). On multivariate analysis high intensity tumour budding had no statistically significant association with positive lymph nodes or prognosis ($p = 0.49$) unlike in the previous studies [9–13]. The reason for this finding may be because the presence of tumor budding (i.e. marked and wide spread cellular dissociation in small groups or in single cells) itself indicates Group 2 worst POI. Hence dividing it into high and low intensity groups has no added advantage in GBCSCC in contrast to previous studies.

In our study, univariate analysis showed that Tumor thickness >5 mm ($p = 0.009$) and positive lymph nodes ($p = 0.01$) had significant correlation with treatment failure and reduced survival. In the adjusted multivariate analysis also Tumor thickness >5 mm ($p = 0.009$) and

Fig. 2 **a** Strands of infiltrating tumor cells (Group 2 worst POI). **b** Islands of tumor cells (Group 2 worst POI) on low power. **c** Diffuse infiltrative front (Group 2 worst POI) with *arrow heads* showing tumor budding (Isolated single cells or small cell clusters of <5 cells) on high power (40×)

positive lymph nodes ($p = 0.01$) retained their strong association with treatment failure and decreased DFS in GBCSCC regardless of the tumor stage, size, differentiation, lymphovascular or perineural invasion (Tables 3 and 4). Our observations are in accordance with previous reports [20–22] and raise the question if multimodality treatment (adjuvant radiation) should be considered in this group of patients with tumor thickness >5 mm.

Conclusion

In conclusion, the results of our prospective study confirm the predictive value of worst POI for positive lymph nodes in patients with GBCSCC. Tumor thickness >5 mm and positive

lymph nodes are independent negative prognostic factors. Because diffuse infiltrative/worst POI correlates with positive lymph nodes, it indirectly influences survival. Our classification of POI into 2 groups is less confusing and easy to reproduce and it can be easily and rapidly analyzed from the routine H & E stained histopathological examination. Tumour budding had no statistically significant association with lymph nodal metastases or prognosis in GBCSCC.

Limitations

Our study group included only GBC subsite of oral cancer and has relatively small number of patients and may not be representative of other populations.

Fig. 3 Perineural invasion seen on oil immersion (100×)

Future Directions

The various histopathologic factors reported in literature as reliable parameters for determining regional metastases are not available preoperatively and so cannot be used to decide on regional treatment. A detailed evaluation of POI on pre-operative diagnostic biopsies might help in identifying patients at higher risk of having lymph node metastasis and thus planning definitive treatment for neck.

A comprehensive understanding and assessment of the POI at the ITF in all subsites of oral and Head & Neck cancer may lead to appropriate treatment planning and may be a simple step towards individualized patient management.

Furthermore, should we consider the use of multimodality treatment, including radiation and/or chemotherapy, in the group of patients with tumor thickness >5 mm needs to be answered?

Conflict of interest No conflict of interest declared.

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