International Journal of Research in Medical Sciences
Mohan KR et al. Int J Res Med Sci. 2019 Oct;7(10):3854-3861
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20194321

Original Research Article

Tumor budding and depth of invasion can be used as prognostic risk factors in determining treatment plan for early stage oral squamous cell carcinoma

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Received: 09 August 2019
Accepted: 05 September 2019

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ABSTRACT

Background: The grading of oral squamous cell carcinoma can be useful along with TNM staging in determining treatment plan. The aim is to evaluate the prognostic value of histopathological grading of oral squamous cell carcinoma and to find its importance in setting appropriate treatment plan.
Methods: The study includes 60 oral squamous cell carcinoma cases surgically operated during January 2012 to December 2018. From the archival paraffin blocks and available resected specimens of each case, the histological parameters used in Bryne's invasive grading system and Almangush BD model were evaluated and compared to their prognosis.
Results: The parameters used in BD model-tumor budding and depth of invasion were found to be statistically significant with prognosis of the disease. Except for nuclear polymorphism, the parameters used in Bryne's invasive front grading system do not correlate with prognosis.
Conclusion: Based on the prognostic significance, tumor budding ≥5 buds in the invasive front area and depth of invasion ≥4mm can be used as risk factors in prospective clinical trials by considering them in early stage disparity cases for multimodality treatment approach and elective neck dissection.

Keywords: Depth of invasion, Elective neck dissection, Histopathological parameters, Oral squamous cell carcinoma, Tumor budding

INTRODUCTION

Oral cancer is one of the most common cancers in the world affecting both men and women especially in the South-east Asian countries like India, Bangladesh, Sri Lanka, Thailand, Indonesia and Pakistan of which majority is squamous cell carcinoma. The main reason behind the enormous prevalence of oral cancer may be suggested due to habits among the population such as betel quid chewing, snuff dipping and reverse smoking. Clinical staging of the disease based on TNM classification and location of the tumor are routinely considered as the main criteria to determine the prognosis and treatment.[1] However, variations in the treatment response and prognosis are high for oral squamous cell carcinoma, with some patients having prolonged survival, while others may die of metastasis rapidly even though the site and the stage are same. Despite the advances in multimodality treatment protocol, the survival rate amongst the affected population during late stages

remains low. It has been elucidated by authors that a detailed histopathologic grading could help the clinicians to assess the prognosis and determine a suitable treatment plan, which will eventually benefit the outcome.[2-7]

Initially, the histopathological grading for oral squamous cell carcinoma developed by Broders in 1920, then adopted by World Health Organization (WHO) is universally followed taking into account the degree of keratinization and levels of cellular and nuclear pleomorphism, but found to be of little prognostic value which proposed a need for a better grading method.[3-5] Later, Anneroth et al. (1987) developed multifactorial grading system that includes pattern and depth of invasion and host tissue response.[3] Bryne et al. (1992) suggested a hypothesis that the molecular and morphological characteristic at the invasive front area of oral squamous cell carcinoma reflects prognosis better than the other parts due to tumor heterogeneity.[4] Finally, to make it simple, Almangush et al. (2015) introduced BD model which considers only tumor budding and depth of invasion in the invasive front area that proved to have significant correlation with the prognosis.[7] The grading of tumors is more precise in the surgically resected tumors as they show correct invasive front areas by grossly examining the specimen and studying their appropriate tissue sections. Moreover, disparity usually exists in determining the treatment plan for early stage oral cancers where surgery is primary option, either to treat only by single modality (surgery alone) or to treat with multimodality approach (Surgery+chemoradiotherapy) and when to consider elective neck dissection (END).

Selecting the appropriate treatment modality is utmost important as any clause will be detrimental to the patient during long term either in the form of recurrence, residual lesion or permanent morbidity. Many recent studies have shown that once the primary treatment fails then the death rate due to disease is exponentially increased and chance of survival is questionable.

The recent National Comprehensive Cancer Network (NCCN-2018) guidelines for management of early stage oral cancer considers histopathological parameters such as- two or more nodal metastasis, margin positivity, cervical node metastasis with extra-capsular extension, perineural or lymphovascular invasion and nodal involvement in level 4 and 5 region as adverse risk factors and recommends adjuvant therapies based on trials in USA and Europe.[12] The grading systems has been studied and utilized in oral squamous cell carcinomas of Western population but not in high prevalence countries like India where the primary etiology is different. The aim of the study is to evaluate the prognostic significance of histological parameters used in Bryne's malignancy grading system and Almangush BD model in the oral squamous cell carcinomas affecting Indian population and to guide clinicians in selecting appropriate treatment plan.

METHODS

A total of 60 oral squamous cell carcinoma cases surgically operated at Government Arignar Anna Cancer Hospital, Regional Cancer Center during January 2012 to December 2018 were selected. Inform consent from each patients and ethical approval for the study was obtained. The Inclusion criteria were the patients with oral squamous cell carcinoma who were treated curatively with surgery as a primary modality. Adjuvant therapies with chemoradiation were given to 34 patients based on the staging of disease and according to NCCN (2018) guidelines. The exclusion criteria were- the patients who were treated by chemoradiation/ Pre-op chemoradiation as initial modality, the patients who had undergone brachytherapy prior surgery, the patient who were treated either by surgery or chemoradiation in form of palliative intend, the patients with distant metastasis during initial visit, the patients who did not have adequate follow-up information, the patients with/without co-morbidities who expired form other causes apart from oral squamous cell carcinoma. Form the included 60 patients; there were 36 males and 24 females and the age in between 28 to 75 years. All of them had adequate case data and follow-up records ranging from 28 weeks to 334 weeks with mean 74 weeks follow up details. The archival paraffin bocks and available resected specimen of the selected cases were retrieved from the Department of Pathology. After carefully examining the resected specimens and their corresponding Hematoxylin and Eosin (H&E) stained slide sections, the sections showing tumor with deep invasive area were included in the study. The clinical data and follow-up records were obtained from the database available in the Medical Records Department (MRD) and the current status was obtained by reviewing them in the Department of Dental Surgery. The current status, survival status and prognosis of the patients were recorded. The patients were categorized into (a)- Patients with good prognosis having disease free state on follow up (GP) and (b)- Patients with worst prognosis having either locoregional recurrence or residual disease, distant metastasis or death due to disease (WP). The patients who expired on other causes were not included in WP category.

Malignancy Grading

Histopathological malignancy grading was performed on 4-µm H&E stained sections based on the criteria described by Bryne et al and Almangush BD et al. The Bryne's invasive front grading system includes 5 morphological parameters: degree of keratinization, nuclear polymorphism, number of mitosis per high power microscopic field, pattern of invasion and lymphoplasmacytic cell infiltrate which were score in the scanner view (magnification x 40) from 1 to 4 according to the level of severity (Table 1). The grading was performed independently by two pathologist and inter-observer agreement and reproducibility were reached with a Kappa coefficient acceptable for clinical use. The

Almangush BD model includes tumor budding and depth of invasion as morphological parameters. Tumor budding is defined as the presence of a single cancer cell or small cluster of <5 cancer cell at the invasive front. The depth of invasion was measured from tumor surface to the deepest point of invasion. In cases of verrucous growth, the depth of invasion was measured from the adjacent surface epithelium to the deepest point of invasion. The cut-off value for tumor budding was set at 5 buds (low <5; high ≥5) and cut-off point for depth of invasion was set at 4mm (low <4mm and high ≥ 4mm). Both the parameters of BD model were examined in scanner view (magnification x40) with scores ranging from 0 to 2 were given by adding the scores on tumor budding and depth of invasion (Table 1).

Table 1: Histopathological parameters used for grading oral squamous cell carcinoma.

I. M Bryne Histological Grading System				
Morphological Parameters	Score			
	1	2	3	4
Degree of keratinization	Highly keratinized (>50% of cells)	Moderately keratinized (20-50%of cells)	Minimal keratinization (5-20% of cells	No keratinization (0-5% of cells)
Nuclear polymorphism	Little nuclear polymorphism (>75% mature cells)	Moderately abundant nuclear polymorphism (50-75% mature cells)	Abundant nuclear polymorphism (25-50% mature cells)	Extreme nuclear polymorphism (0-25% mature cells)
Number of mitosis (per High power filed)	0-1	2-3	4-5	>5
Pattern of invasion	Pushing, well-delineated infiltrating borders	Infiltrating, solid, cords, bands and/or strands	Small groups or cords of infiltrating cells (n>15)	Marked and widespread cellular dissociation in small groups and/or in single cells (n<15).
Lymphoplasmacytic infiltration	Marked (prominent rim of inflammatory cells)	Moderate (large clusters of inflammatory cells)	Slight (small irregular clusters of inflammatory cells)	None
Total Bryne Score: Low risk (Score 5-8), Intermediate risk (Score 9-12), High risk (Score 13-20).				
II. A Almangush BD model				
BD score	Histological description			
Score o	Tumor with <4mm depth of invasion and <5 buds at the invasive front			
Score 1	This tumor should have only one of the following features: Tumors with ≥4mm depth of invasion and <5 buds at the invasive front, OR Superficial tumor <4mm, but with high activity of tumor budding at the invasive front (≥5 buds)			
Score 2	Tumors with ≥4mm depth of invasion and with high activity of tumor budding at the invasive front (≥5 buds)			
Total BD score: Low risk Score 0, Intermediate risk Score 1, High risk Score 2.				

Statistical Analysis

The Pearson Chi-Square test and Fisher's Exact Test was used to find the correlation of different clinicopathological variables with the current status and prognosis of disease.

The analysis was done using IBM SPSS statistical software and p values <0.05 were considered significant. Multinomial regression analysis was performed to find the importance of histological variables. Kaplan-Meier Analysis was utilized to know the survival rate.

RESULTS

The clinical distribution of the cases has shown in the (Table 2). The commonest site of oral squamous cell carcinoma is buccal mucosa (40%) followed by tongue (35%). The parameters such as clinical tumor size, nodal status, pathological tumor size and pathological nodal involvement has showed significant correlation with the prognosis of the disease (Table 2). As the size of the primary tumor increases the lymph node metastasis occurs considerably (p=0.001) with 25.5% of T2 size, 61.5% of T3 and all the T4a tumors showing lymph node metastasis.

Table 2: Clinical distribution of oral squamous cell carcinoma cases with prognosis.

Variables	Number of patients	Percentage	Prognosis (p-value)
Gender			0.07
Male	36	60	
Female	24	40	
Clinical Site			0.061
Buccal Mucosa	23	40	
Tongue	21	35	
Lower alveolus	5	8.3	
Lower Lip	3	5	
Floor of mouth	2	3.3	
Upper alveolus	1	1.7	
Clinical Size			0.016
cT1	12	20	
cT2	32	53.3	
cT3	13	21.7	
cT4a	3	5	
Clinical Node			0.037
cN0	44	73.3	
cN1	12	20	
cN2	3	5	
cN3	1	1.7	
Grade			0.087
Well Differentiated	47	78.3	
Moderately Differentiated	11	18.3	
Poorly Differentiated	2	3.4	
Pathological Size			0.047
pT1	32	53.3	
pT2	23	38.3	
pT3	5	8.3	
Pathological Node			0.082
pN0	41	68.3	P value
pN1	9	15	with size of tumor
pN2	10	16.7	P=0.001
Resected Margin status			0.071
Free	54	90	
Involved	6	10	
Degree of differentiation			0.901
Score-1	43	71.7	
Score-2	15	25	
Score-3	2	3.3	
Score-4	0	0	
Nuclear Polymorphism			0.015
Score-1	30	50	
Score-2	22	36.7	
Score-3	8	13.3	
Score-4	0	0	
Mitosis(High power field)			
Score-1	40	66.6	
Score-2	18	30	0.757
Score-3	2	3.3	
Score-4	0	0	
Pattern of Invasion			0.213
Score-1	15	25	

Score-2	15	25	
Score-3	13	21.7	
Score-4	17	28.5	
Lymphoplasmacytic infiltration			0.104
Score-1	41	68.3	
Score-2	12	20	
Score-3	7	11.7	
Score-4	0	0	
Total Bryne Score			
Low	41	68.3	0.295
Intermediate	23	38.3	
High	2	3.4	
Tumor Budding			0.007
Score 0	41	68.3	
Score 1	19	31.7	
Depth of Invasion/Thickness			0.000
Score 0	21	35	
Score 1	39	65	
BD Score			0.000
Score 0	19	31.7	
Score 1	24	40	
Score 2	17	28.3	

The usual site of operable-T4a tumors was lower alveolus having predominate lymph node metastasis. The other sites for frequent lymph node metastasis were buccal mucosa (43.4%) and tongue (28.5%).

Broders grading of whole tumor had no relation in terms of prognostic value. (p=0.087).

The univariate analysis using Pearson chi-square test and Kendil correlation has shown nuclear polymorphism (p=0.015) was a significant factor that correlates with prognosis of the disease but the other parameters used in Bryne's invasive front grading system did not show any significant correlation including the total Bryne's score (Table 2).

The parameters given by Almangush BD model- tumor budding and depth of invasion were found to be highly significant with the prognosis of the tumor (p<0.01). 19 oral cancer cases (31.6%) showed more than 5 buds in the invasive front area (Figure 1D), 39 cases (65%) showed depth of invasion more than 4mm depth (Figure 1C and 1E) and 17 cases (28.3%) showed both high tumor budding and depth of invasion more than cut off value (Figure 1E and 1F). The tumors occurring in the lower alveolus had higher rate of tumor budding and increased depth of invasion (high-risk BD score), which leads to higher chance of nodal metastasis and worst prognosis.

The tumors occurring in the lower lip showed low-risk BD score (Figure 1A and 1B) and had good prognosis.

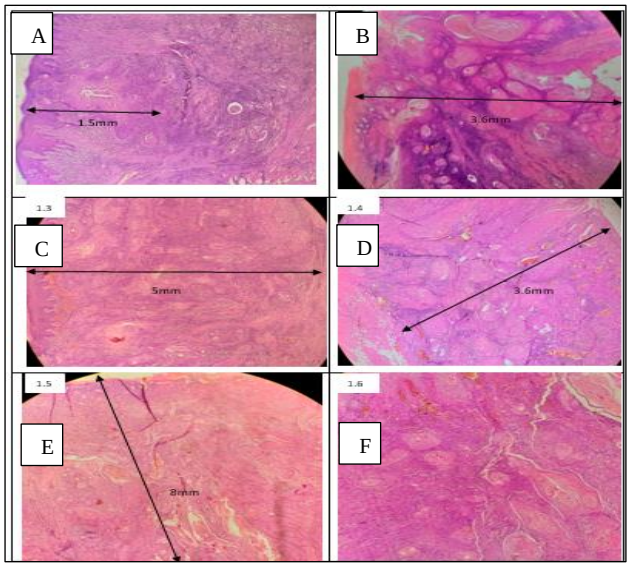

Figure 1: (A) H and E Section shows tumor with depth of invasion 1.5mm, BD score-0[x40]. (B) H and E Section shows tumor with depth of invasion 3.6mm with keratin pearls and no separate tumor buds, BD score-0 [x40], (C) H and E Section shows tumor with depth of invasion 5mm but no tumor budding, BD score-1[x40], (D) H and E Section shows tumor with depth of invasion 3.6mm with tumor budding more than cut off value of 5 buds, BD score-1 [x40], (E) H and E Section shows tumor with depth of invasion 8mm also with tumor buds more than 5, BD score-2 [x40], (F) H and E Section of the same tumor in the picture (E) showing more tumor buds in the invasion area [x40].

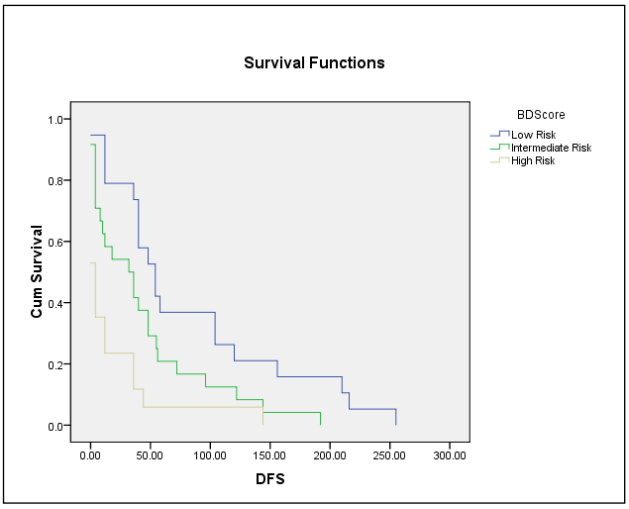

Figure 2: Kaplan-Meier curves showing comparison between disease free state (DFS) in weeks and corresponding BD scores (low risk, intermediate risk and high risk).

In the logistic multinomial regression analysis, high-risk BD score which denotes the tumors having both high budding and increased depth of invasion showed twice the possibility of developing worst prognosis when compared to other tumors with 95% confidence interval.

According to kaplan-meier survival analysis, high-risk BD score is significantly related to the death due to disease (Log Rank p<0.001). Disease free survival (DFS) in weeks was shown in comparison with BD scores (Figure 2).

DISCUSSION

The current treatment paradigms for oral squamous cell carcinoma cases is predominantly based on TNM staging and patient performance status, but their prognosis remains controversial especially in early stage disease where some perform well with single modality but others may require extensive management. In fact the disparity usually occurs when to perform multimodality treatment for early stage cancers by keeping in mind the complications associated and also there are no clear guidelines as when to perform elective neck dissection (END). Some may consider imaging techniques and sentinel node navigation but there are no adequate supportive evidences to claim its veracity. In addition, the complexity of lymphatic drainage in head and neck region usually make the detection of sentinel lymph node in oral squamous cell carcinoma difficult and time consuming. Moreover, the establishment and cost for these procedures are extensive in usual setup. Hence, to complement the shortcomings of TNM staging, histological grading of tumors was used in previous studies as a reliable method in designing precise treatment plan.[4-9] Grading of tumors is more appropriate in the surgically resected tissues, which is still the cornerstone in routine clinical practice.[4]

Broders conventional histopathological malignancy grading system, which is routinely followed by many diagnostic centers, were found to be insignificant similar to the previous studies.[4] implying its poor prognostic value. Invasive front malignancy grading system introduced by Bryne et al emphasizing the importance of grading deep invasive front region showed prognostic significance in studies.[1,5] They have conveyed that due to tumor heterogeneity the deepest part of the tumor will be more dedifferentiated and also proved by cross examining with decrease in expression of membrane bound carbohydrate and blood group antigen H.

The grading in this study was done by duly examining the resected specimens and their corresponding slide sections to appreciate invasive front area precisely. The inter- and intra-observer variations were present in the selecting the appropriate fields and scoring the first 3 parameters of the Bryne's system: degree of differentiation, nuclear polymorphism and the mitotic count. Although an acceptable agreement was reached based on kappa coefficient, Bryne illustrated high significant results and reproducibility could be obtained by omitting mitotic index. However, we could find the kappa coefficient increased adequately by considering the last 2 parameters of Bryne's system: pattern of invasion and lymphoplasmacytic infiltrate only and by omitting all the other parameters. In our study Bryne score were found to be insignificant in determining the prognosis (P=0.295), which contradicts the previous studies.[1,4] may be due to the differences in field selection and inter and intra observer variability. Hence, utilizing this grading system and its parameters will be of questionable efficacy.

BD model which utilizes evaluation of tumor budding and depth of invasion was found to be highly significant with the prognosis of disease (P< 0.01). This model can be used as a risk factor in considering END and multimodality treatment approach in disparity cases. In multivariate analysis it is found that high BD score was a significant factor and denotes twice chance of developing worst prognosis. Disease Free State (DFS) in weeks were calculated and comparison with BD score showed an obvious connection which can be appreciated in the graph (Figure 2).

Grading of fresh frozen specimen using BD model during the time of surgery may be helpful in considering elective neck dissection. From a biological point of view, the BD model deals with depth of tumor invasion, which indicates the efficacy of tissue penetration, and also measures tumor budding, which reflects epithelial-mesenchymal transition (EMT) and tumor dissociation at invasive front. Tumor budding also depends upon the density of stromal myofibroblasts.[7]

In this study depth of invasion (p<0.001) emerged as the highly significant prognostic factor and also associated with nodal metastasis (p=0.03) similar to the Larsen et al, Hung et al, and Kurokawa et al.[14,20,23] Tumor depth is measured from the surface of the tumor to the deepest

point of invasion (Figure 1). Several authors have found that the critical thickness for oral squamous cell carcinoma developing nodal metastasis is 4mm.[15,16] In this study, 39 cases showed depth of invasion more than 4mm of which 19 cases (48.7%) showed nodal metastasis when compared to single case of nodal metastasis in tumors with less than 4mm depth. When considering the definition of depth of invasion, differences exists whether to consider measurement form: (a)- the surface/base of the ulcer to the deepest point of invasion, (b)- from adjacent intact mucosa to the deepest point of invasion, (c)- from basement membrane to deepest point of invasion. Previous studies have taken different reference points to measure the depth of invasion.[21,22] In this study we measured depth of invasion from surface of the tumor to the deepest point of invasion to make it simple and easily reproducible (Figure 1). Depth of invasion may also be influenced by the shrinkage due to fixation. The variation in calculating depth of invasion is high in verrucous growth by over-estimating, so in such cases the reference point was considered form adjacent mucosa enabling appropriate measurement. Depth of invasion or tumor thickness is already a feature in staging of melanomas, endometrial carcinomas and cervical cancers. In addition, the depth of invasion based on anatomical layers was also utilized in staging of esophagus, stomach, colon and rectal cancers. The eight edition of American joint cancer committee (AJCC-2018) for staging of oral cancers has incorporated depth of invasion (DOI) as criteria and by upstaging the tumors with DOI greater than 5mm. The result of this study has shown that depth of invasion clearly influences the clinical outcome of the patients with oral cancer and a reliable predictor for lymph node metastasis.

The tumor budding (p=0.01) is also a significant factor in determining the prognosis and survival of oral cancer patients but not the nodal metastasis (p>0.1) making END optional. In our study, 19 cases (31.6%) showed high tumor budding more than the cut-off value in the invasive front area, amongst those (except two) all had eminent depth of invasion greater than 4mm. The cancer cells located in the invasive tumor front are more aggressive in terms of metastatic potential and utilized in various studies to determine its prognostic significance. The cancer cells in this region have the potential for morphologic switch characterized by epithelial-mesenchymal transition and associated with increased cell mobility and invasiveness.[17] It has been previously demonstrated as a valuable prognostic factor in breast, lung, esophageal and colorectal cancers.[28-30] The reduction in the survival rate was found to be significant with tumor budding in accordance to the studies.[17] At times the small buds or dispersed cells may be difficult to identify especially in poorly differentiated squamous cell carcinomas due to cancer cell morphology and surrounding lymphocytic infiltration. In that situation it may require immunohistochemistry staining with pancytokeratin. Recently, tumor budding in oral squamous cell carcinomas has been evaluated using digital imaging to show better accuracy and reproducibility.[27]

CONCLUSION

Although the prognosis depends upon many factors including clinical and pathological stage, treatment options and patient performance, authors could find supportive evidences for both tumor budding ≥5 buds in the invasive front area and depth of invasion ≥4mm as prognostic markers and recommend being followed in prospective clinical trials. Authors suggest the clinicians to consider these parameters as risk factors and to proceed with multimodality treatment and elective neck dissection in early stage disparity cases. However, tumors having budding phenomenon without eminent depth of invasion may not be considered as risk factor. These parameters are simple, cheap, reproducible and universally available, better than traditionally followed grading system.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee of Govt. Arignar Anna Cancer Hospital, Regional Cancer Center, Kanchipuram, Tamil Nadu, India-631552, Ref.No.5163/ME/2017-2 dated 29-12-2017.

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Cite this article as: Mohan KR, Sharief RM, Rahila C. Tumor budding and depth of invasion can be used as prognostic risk factors in determining treatment plan for early stage oral squamous cell carcinoma. Int J Res Med Sci 2019;7:3854-61.