

Histological assessment of budding and depth of invasion (BD) model in biopsies of oral squamous cell carcinoma

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Abstract

Objectives: Recognizing precise prognosticators from preoperative biopsies that aids in treatment is of immense clinical importance. Thus, the aim of this study was to assess and compare the tumor budding (B), depth of invasion (D) and combined scores (BD) model in the preoperative biopsies and subsequent postoperative specimens of oral squamous cell carcinoma (OSCC).

Material and Methods: B and D were assessed in the pre- and postoperative specimens of 65 OSCC cases treated in the institution. Relationship between pre- and postoperative assessments was subjected to McNemar's, Chi-square, Fisher's exact, sensitivity and specificity statistics.

Results: There was an agreement between the pre- and postoperative B scores in 54 cases with accuracy of 83% (95% confidence interval 71.73%–91.24%). The preoperative scores showed a good sensitivity of 67.86% and a high specificity of 94.59% in predicting the postoperative score of the same. The difference in assessing intensity B as low and high between preoperative and postoperative biopsies was not statistically different. There was an agreement between the pre- and postoperative scores of the BD model in 72%. The difference in BD scoring as low intermediate and high between preoperative and postoperative biopsies were significantly different statistically. Postoperative BD scoring showed a significant association with stage and lymph node metastasis.

Conclusions: The findings validate the prognostic value of BD model in the postoperative specimens. Its value in preoperative biopsies is questionable. A judicious representative biopsy may increase the accuracy and reliability in the assessment of preoperative B and precision in BD model evaluation.

Keywords: BD model, depth of invasion, oral squamous cell carcinoma, tumor budding

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INTRODUCTION

Histopathological prognostication of oral squamous cell carcinoma (OSCC) has been based primarily on the classical parameters such as tumor grade, status of

surgical margins, depth of invasion, lymphovascular invasion, perineural invasion, host response and mitotic activity which are regularly assessed in hematoxylin and eosin (H&E)-stained sections. Such information is added

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in the reports to assist in forecasting the behavior of OSCC.^[1,2] Current reports have identified the presence of tumor budding activity, defined as small groups of tumor cells,^[3] as a prognosticator indicating an unfavorable outcome in OSCC patients.^[4,5] Tumor budding (B) represents the group of cancer cells that are more invasive than cells in the main tumor mass. It is a specific type of invasive growth in carcinomas characterized by invading single tumor cells or small clusters of tumor cells (<5 cells) at the invasive tumor front (ITF).^[2] B initially named as “sprouting” by Imai^[6] Morodomi *et al.* were the first to report the significance of B on biopsy samples in relation to lymph node metastasis (LNM). The oncologic significance of B along the ITF was first described in colorectal carcinoma (CRC) and was suggested to correlate with higher malignant potential and biologic aggressiveness of the tumor.^[7] The presence of B has been associated with aggressive behavior of the tumor and has been correlated with LNM, recurrence, distant metastasis and decreased survival in CRC, esophageal carcinomas, lung carcinoma and so on.^[8-10] Thus, B is an important parameter for the assessment of tumor behavior. The frequency of B varies widely in literature in various cancers (20%–89%).^[8] Its value as a prognostic marker in head-and-neck squamous cell carcinoma (HNSCC) has been analyzed only recently.^[2] The prognostic significance of B, apparently simple to evaluate the parameter is sparse in OSCC with the few existing studies concentrating predominantly on oral tongue squamous cell carcinoma (OTSCC).^[11-13] Few researchers have studied the presence and the prognostic significance of B in OSCC. The prognostic value of B assessed in resection specimens in OSCC is gaining acceptance,^[1] the value of B in preoperative biopsies, which normally do not encompass the ITF has not been thoroughly investigated yet. Most recently, Almangush *et al.* and Seki *et al.* have cited the importance of assessment of B in preoperative biopsies of OSCC.^[14,15] Seki *et al.* found that B evaluated using biopsy specimens was a good predictive factor for LNM in SCC of the tongue and floor of the mouth. In addition, there was a good correlation between biopsies and resected specimens for B.^[16]

The utilization of single or combination of histopathological attributes for the prognostication has been initiated in the number of reports. Histological assessment of biopsies is still the basis of pathological grading in regular clinical practice.^[13] A combination of two attributes will increase the accuracy and reliability of prognostication. One such representation is the BD model, which deals with depth invasion (D), which specifies the tissue infiltration, and the model as well evaluates B, which reveals the epithelial mesenchymal transition (EMT) and tumor

cell dissociation at ITF.^[14] The prognostic value of BD model on postoperative biopsies has been validated in the cohorts of OSCC.^[17] Recently, Seki *et al.* and Almangush *et al.* assessed B and D even in the preoperative biopsies of OTSCC.^[14,16] Almangush *et al.* found that the BD scores significantly corresponded to the scores of postoperative tumor resection samples and a BD model can be assessed in a satisfactory biopsies of OTSCC.^[14] The BD model has not been investigated in preoperative and corresponding postoperative biopsies of OSCC in a considerable cluster. Recognizing precise prognosticators from preoperative biopsies that aids in the treatment is of immense clinical importance. Hence, the aim of the present study was to assess and compare the intensity B, D of tumor and combined BD scores in preoperative biopsies and the corresponding postoperative excision samples of OSCC.

MATERIALS AND METHODS

H&E-stained sections from incisional and corresponding excisional biopsies of 98 OSCC subjects diagnosed and treated in the craniofacial unit (CFU) of our institution between the years 2014–2018 were retrieved for this analysis. The study protocol was approved by the Institutional Ethics Committee SDMCDSh-IEC (IRB·No. 2015/S/OP/37). For all cases, the clinical diagnosis was confirmed based on incisional biopsy report and were treated by surgical excision along with neck dissection. OSCC cases only in which both pre and corresponding postoperative sections were available along with relevant details were included in the study. A total of 65 cases met the inclusion and exclusion criteria and were subjected for the comparative analysis. Preoperative biopsies taken from the edge of tumor, with an inadequate representative area, insufficient volume and with artefacts were excluded. Patients with preoperative chemotherapy or radiotherapy, with multiple recurrence were excluded; cases with histologic variants of SCC such as verrucous, basaloid and spindle cell carcinoma were also excluded.

The tumor stage was defined according to the TNM classification of the UICC. In addition, other clinical features such as age, sex, site and size of tumor were also obtained from the patients’ file retrieved from the archives of CFU of the institution.

B was defined as the presence of single cancer cell or cluster of less than five cancer cells. The invasive front (IF) was initially scanned with ×4 objective lens, and then, the field with the highest density of B was counted using ×20 objective lens. The presence of B within the whole tumor mass was also considered in the preoperative samples.

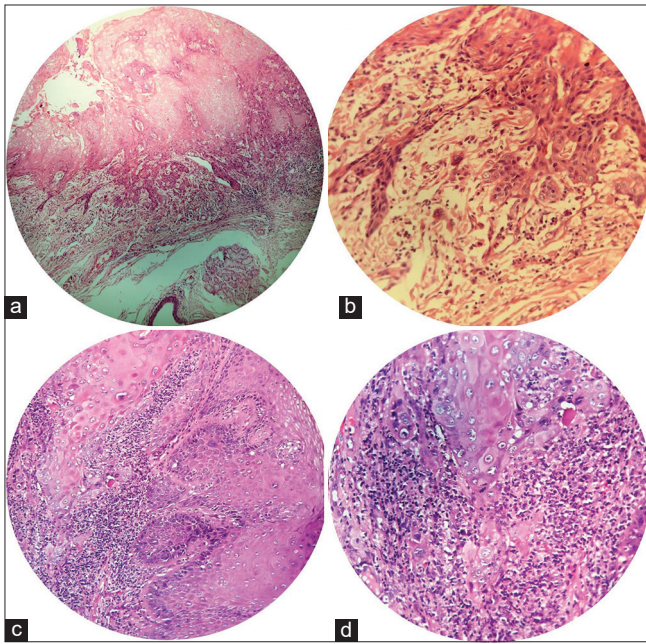


Figure 1: Photomicrograph (a-d) depicting low-intensity B in the preoperative biopsies (H&E, $\times 5$, $\times 20$, $\times 40$)

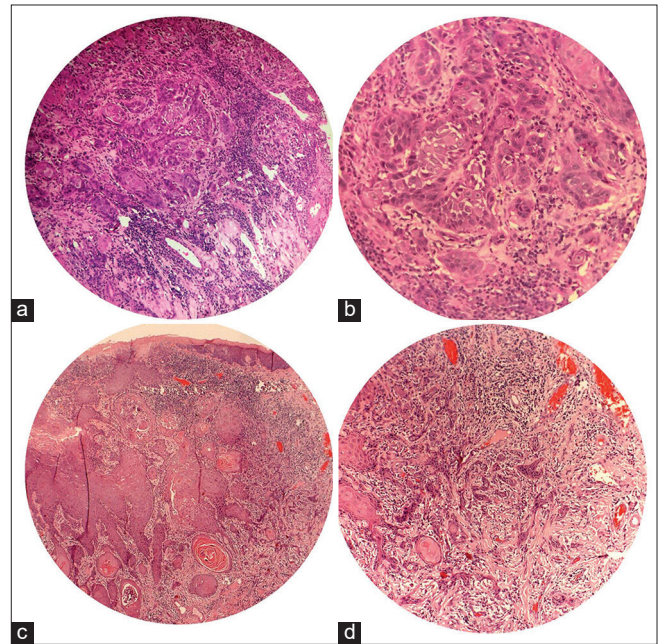


Figure 2: Photomicrograph (a-d) depicting high-intensity B in the preoperative biopsies (H&E, $\times 5$, $\times 20$, $\times 40$)

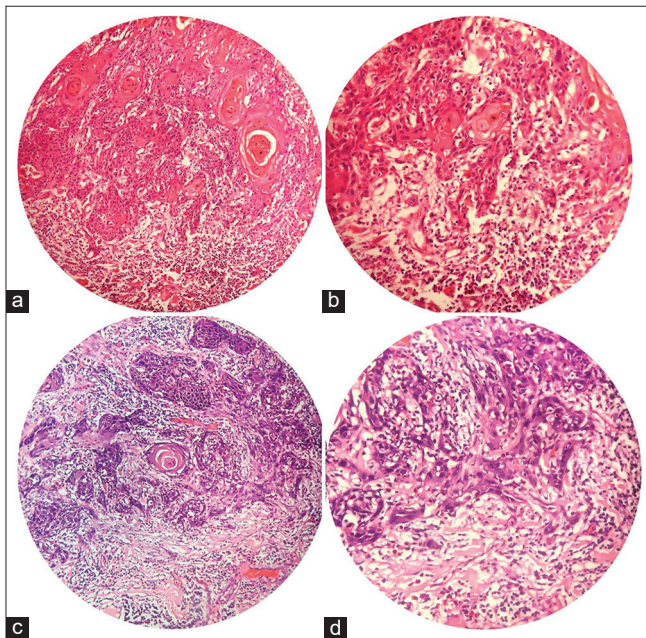


Figure 3: Photomicrograph (a-d) depicting low-intensity B in the postoperative biopsies (H&E, $\times 5$, $\times 20$, $\times 40$)

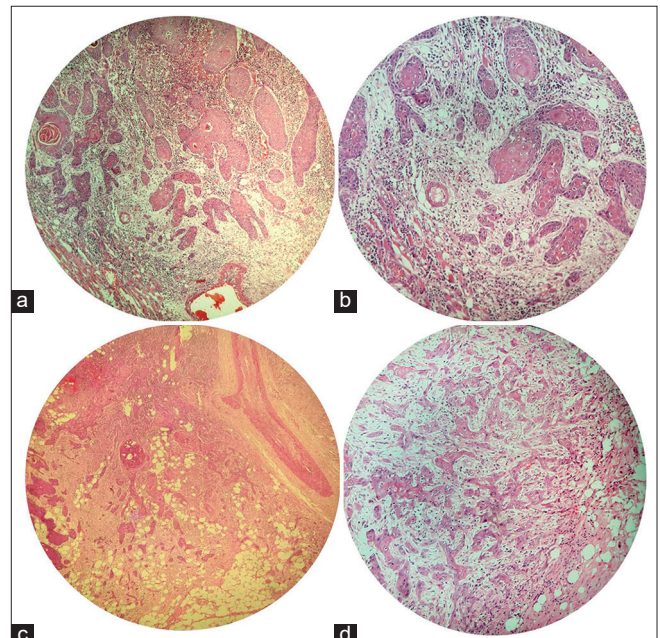


Figure 4: Photomicrograph (a-d) depicting high-intensity B in the postoperative biopsies (H&E, $\times 5$, $\times 20$, $\times 40$)

D was measured from the surface of the tumor to the deepest point of invasion. All the samples were analyzed by two observers who followed uniform criteria and were blinded to the final outcome. Before the commencement of the principal study, a pilot study was conducted to assess intra and interobserver consistency. BD scores were assigned as previously described. In brief, score 0 refers to < 5 buds at the IF and < 4 mm in depth. Score 1 refers to either the presence of ≥ 5 buds at the IF or a deep tumor

of ≥ 4 mm in depth. Score 2 refers to the presence of ≥ 5 buds at the IF and a deep tumor of ≥ 4 mm in depth.^[13,14]

All analyses were performed with IBM SPSS version 20 (IBM Corp., Armonk, NY, USA). The statistical significance of the relationship between pre- and postoperative measures was evaluated using the McNemar's test and Chi-square test. For sensitivity and specificity statistics with their 95% confidence intervals (CIs), BD scores of

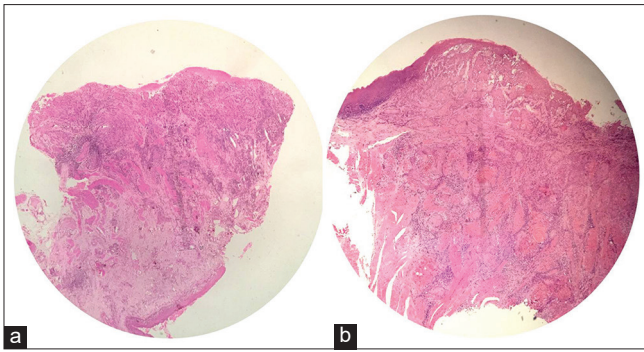


Figure 5: Photomicrograph (a and b) demonstrating superficial and deep tumors in the preoperative biopsies (H&E, $\times 1.25$)

low and intermediate were combined together (low and intermediate vs high) to evaluate the predictive power of preoperative score for the postoperative score of the corresponding sample. The association between the preoperative and postoperative budding index, depth, BD scores and clinicopathological parameters were examined using the Chi-square and Fischer's exact tests. $P < 0.05$ was considered statistically significant.

RESULTS

Of the 98 OSCC cases diagnosed and treated in the CFU of our institution from 2014–2018. Only 65 cases met the inclusion and exclusion criteria and were evaluated. There were 54 males (83%) and 11 females (17%). Stage distribution was as follows: 8 cases (12%) were assigned as Stage I, 31 cases (48%) as Stage II, 18 (28%) as Stage III and 8 cases (12%) as Stage IV. The mean age at the diagnosis was 52 years (range 26–82 years). Sixty-five percent of cases had the lesion involving the buccal mucosa, gingivobuccal sulcus and retromolar trigone, 11% lateral border of the tongue, 7% lip and 17% palate and maxillary alveolus. Forty-five (69%) cases were without LNM and 20 cases (31%) were with LNM.

The number of B in preoperative biopsies ranged from 0 to 20 buds (median 3 and mean of 4.8), and the corresponding postoperative samples ranged from 0 to 42 buds (median 4 and mean of 9.75). Of the cases, 54 had the same B category (low < 5 buds or high 5) in the pre and postoperative samples [Figures 1-4]. There was an agreement between the preoperative and postoperative B scores in 54 cases with accuracy of 83% (95% CI 71.73%–91.24%). The preoperative scores showed a good sensitivity of 67.86% (95% CI 47.65%–84.12%) and a high specificity of 94.59% (95% CI 18.81%–99.34%) in predicting the postoperative score of the same. The difference in assessing intensity B as low and high between preoperative and postoperative biopsies was not statistically

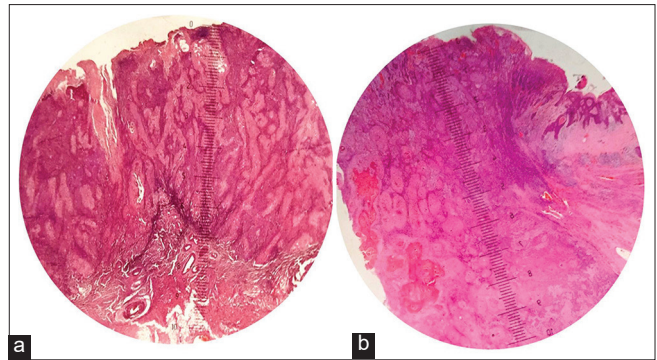


Figure 6: Photomicrograph (a and b) demonstrating superficial and deep tumors in the postoperative specimens (H&E, $\times 1.25$)

different [Table 1]. The association between preoperative and postoperative B was statistically significant ($P < 0.001$).

In preoperative specimens, depth values ranged from 0.8 to 9.8 mm (mean 4.08 mm and median 3.95 mm), and those corresponding postoperative samples ranged from 1.2 to 13mm (mean 6.7 mm and median 6.3mm). Of the cases, 35 had the same D category (superficial < 4 mm or deep ≥ 4 mm) in the preoperative and postoperative samples [Figures 5-6]. There was an agreement between the preoperative and postoperative D scores in 35 cases (54%). The sensitivity for superficial tumor (< 4 mm) was 55.9% (95% CI 42.4%–68.6%), the sensitivity for deep tumor (≥ 4 mm) was 33.3% (95% CI 5.9%–75.8%) and the specificity could not be analyzed as depth cannot be zero. The difference in assessing the D as superficial (< 4 mm) and deep (≥ 4 mm) between preoperative and postoperative biopsies was significantly different statistically. The association between preoperative and postoperative D was not significant ($P = 0.692$).

For the preoperative samples, 43 cases (66%) had BD Score 0, 18 cases (28%) had Score 1 and 4 cases (6%) had Score 2. In the postoperative samples, 27 cases (41.5%) had Score 0, 20 cases (30.7%) had Score 1 and 18 cases (27.6%) had Score 2. There was an agreement between the preoperative and postoperative scores of the BD model in 47 cases. The sensitivity for low-intermediate BD score was 73.7% (95% CI 60.6%–83.2%), the sensitivity for high BD score was 50% (95% CI 9.1%–90.8%) and the specificity could not be assessed, as one of the component of BD, i.e., depth cannot be absent in a tumor. The difference in BD scoring as low intermediate and high between preoperative and postoperative biopsies was significantly different statistically. The association between preoperative and postoperative BD scoring was not significant ($P = 0.305$).

There was no significant association between preoperative B with clinicopathological features ($P > 0.05$). The

Table 1: Distribution of 65 oral squamous cell carcinoma cases according to preoperative and postoperative BD scores

Parameters	Postoperative budding		Total, n (%)	P ^a
	Low (<5), n (%)	High (≥5), n (%)		
Preoperative budding				
Low (<5)	35 (80)	9 (20)	44 (100)	0.065
High (≥5)	2 (10)	19 (90)	21 (100)	
Parameters	Postoperative depth		Total, n (%)	P ^a
	Superficial (<4 mm), n (%)	Deep (≥4 mm), n (%)		
Preoperative depth				
Superficial (<4 mm)	33 (56)	26 (44)	59 (100)	<0.001
Deep (≥4 mm)	4 (67)	2 (33)	6 (100)	
Parameters	Postoperative BD scores		Total, n (%)	P ^a
	Low-intermediate (0-1)	High (2)		
Preoperative BD scores				
Low-intermediate (0-1)	45 (74)	16 (26)	61 (100)	0.001
High (2)	2 (50)	2 (50)	4 (100)	

^aMcNemar test

postoperative B showed significant association with stage, LNM and D [Table 2]. Preoperative BD scores showed significant association with D and B. Postoperative BD scores showed significant association with stage, LNM, D and B [Table 3].

DISCUSSION

B is a histological phenomenon encountered in various cancers, whereby individual malignant cells and/or small clusters of malignant cells are seen in the tumor stroma. They are non-proliferating, nonapoptotic and highly aggressive subpopulation of tumor cells that display migratory and invasive capacities.^[18] Assessment of B was first introduced in HNSCC prognostication in 2010.^[19] Cancer cells at the ITF behave aggressively compared with cancer cells in the superficial or central regions of the main tumor mass. In addition, cancer cells at the ITF may undergo EMT, which is an important step in progression of tumor metastasis.^[1] Tumor buds may provide a histologic means for assessment of EMT.^[8] It has been analyzed and reported in the literature that B at the ITF may be involved in the development of metastasis in OSCC. Results of a meta-analysis on evaluation of B at ITB by Almangush *et al.* demonstrated a significant association of B with LNM, and they concluded B as a simple and reliable prognosticator for OSCC.^[1] Angadi *et al.* first described that B as an independent prognostic factor for prediction of LNM in OSCC and found that high-intensity B was significantly associated with LNM and D.^[8] Even in this analysis, postoperative high-intensity B scores showed significant association with stage, LNM and D. This association of B with D has also been confirmed recently by Almangush *et al.*^[20] This substantiates the likelihood of B's capability in defining the biologic aggressiveness of tumor and serving as an indicator for the metastatic potential in OSCC.^[8] Comparable to Angadi *et al.*'s report, in this analysis postoperative B was evident in 89% of cases with 43% of cases demonstrated high-intensity B.

Table 2: Relationship between postoperative B scores and clinicopathological parameters

Parameters	Subcategory	Tumor budding (%)		Total (%)	P
		Low (<5)	High (≥5)		
		Age (years)	<40		
	>40	33 (57.8)	24 (42.2)	57 (87.7)	
Gender	Male	30 (55.5)	24 (45.5)	54 (83)	0.745 ^a
	Female	7 (63.6)	4 (36.4)	11 (17)	
Stage	Early (1+2)	30 (76.9)	9 (23.0)	39 (60)	<0.001
	Advanced (3+4)	7 (26.9)	19 (73.1)	26 (40)	
Lymph node metastasis	Absent	36 (80)	9 (20)	45 (69)	<0.001
	Present	1 (5)	19 (95)	20 (31)	
Depth of invasion	Superficial	27 (72.9)	10 (27.1)	37 (56)	0.005
	Deep	10 (27.1)	18 (72.9)	28 (44)	

^aFisher's exact test, Chi-square test

Attramadal *et al.*^[21] reported 51.7% of high intensity B, Boxberg *et al.*,^[5] 26.1% and Arora *et al.*,^[22] about 39.6% in the postoperative biopsies.

Recently, Almangush *et al.* and Seki *et al.* evaluated the prognostic value of B in biopsy specimens of OSCC.^[14-16] Seki *et al.* found a good relationship between biopsies and resected specimens for B.^[16] In the present analysis of the 65 OSCC cases, there was an agreement between the preoperative and postoperative B scores in 54 cases with accuracy of 83%. The preoperative scores showed a good sensitivity and a high specificity in predicting the postoperative score of the same. The association between preoperative and postoperative B was statistically significant. Similar findings were observed by Almangush *et al.*,^[14] authors conclude that preoperative B assessment may be of great importance from a clinical point of view for treatment planning of OSCC.^[1]

The subjective identification of the deepest invading tumor cell is same for both D and thickness.^[23] Ambrosch *et al.* reported a strong correlation between the D and nodal disease, their study also showed a D of 4 mm to be a valuable cutoff for the occurrence of LNM. They have

Table 3: Relationship between postoperative BD scores and clinicopathological parameters

Parameters	Subcategory	BD scores (%)		Total (%)	P
		Low-intermediate	High		
Age (years)	<40	5 (62.5)	3 (37.5)	8 (12.3)	0.675 ^a
	>40	42 (73.7)	15 (26.3)	57 (87.7)	
Gender	Male	39 (72.2)	15 (27.8)	54 (83)	>0.99 ^a
	Female	8 (72.7)	3 (27.3)	11 (17)	
Stage	Early (1+2)	36 (92.3)	3 (7.7)	39 (60)	<0.001
	Advanced (3+4)	11 (42.3)	15 (57.7)	26 (40)	
Lymph node metastasis	Absent	42 (93.3)	3 (6.7)	45 (69.2)	<0.001
	Present	5 (25)	15 (75)	20 (30.8)	
Budding	Low	37 (100)	0 (0)	37 (57)	<0.001
	High	10 (35.7)	18 (64.3)	28 (43)	
Depth of invasion	Superficial	37 (100)	0 (0)	37 (56)	<0.001
	Deep	10 (35.7)	18 (64.3)	28 (44)	

^aFisher's exact test, Chi-square test

recommended that cancers of the oral cavity and pharynx with a maximum depth of 4 mm or more are at a higher risk for harboring occult metastasis and should be selected for elective nodal dissection.^[24]

In this analysis, there was an agreement between the preoperative and postoperative D scores in only 54%. Almangush *et al.* found an agreement in 77% of OTSCC and attributed poor class of preoperative biopsies, which was taken from the surface without involving IF, as the reason for inaccuracies in D scores. Researchers have suggested that exactness of evaluation of preoperative D scores can be better with imaging and it is an alternative modality in case of inadequate biopsies to score preoperative D.^[14] The difference in assessing the D as superficial and deep between preoperative and postoperative biopsies was significantly different statistically in this analysis. Even Almangush *et al.* and Seki *et al.*, found that D measurements with postoperative specimens were significantly higher than biopsies.^[14,16] Preoperative samples were unable to record depth exactly, as biopsies are usually taken from the periphery of tumor and limited area of tumor.^[14]

The BD model is a simple, predictive model that can be applied easily on routine H&E-stained section was introduced by Almangush *et al.* 2015.^[13,14] This model showed a strong potential to identify aggressive tumors.^[17] The BD model gives a snapshot of tumor invasiveness and its ability to progress or metastasis.^[13] Recently, the prognostic value of BD model has been validated in cohorts of OTSCC, OSCC and carcinoma of lip.^[13,17,25] In the present study, BD scores obtained from resected specimens showed significant association stage and LNM, as observed by Almangush *et al.*^[14]

In this assessment, there was an agreement between the preoperative and postoperative scores of the BD model in 72% and different scores in 28%. The difference in BD

scoring between preoperative and postoperative biopsies was significantly different statistically. Almangush *et al.* found an agreement in 83% of cases and 11% had different scores.^[14] They found a significant association between preoperative and postoperative BD scoring. In this analysis, preoperative and postoperative BD scores differed in 18 cases, and it has been attributed to, insufficient biopsy volume or non-representative sample or artefacts formed while handling preoperative biopsies. A quality biopsy will aid in assessment of the BD model accurately.

CONCLUSIONS

Results of this analysis validate the prognostic value of BD model in postoperative specimens of OSCC. Findings are in accord that BD model is objective, simplest, fast and most effective tool to evaluate the prognosis in OSCC. However, its value in preoperative biopsies is questionable. There was a significant association between preoperative and postoperative biopsies for assessing B, but the discrepancy were with regard to D measurement. Further investigations are required to substantiate the value of BD model in preoperative biopsies of OSCC. Observations of this analysis recommend that a judicious representative biopsy may increase consistency in the assessment of preoperative B and precision in BD model evaluation in OSCC.

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Conflicts of interest

There are no conflicts of interest.

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