

# Correlative study of tumor budding, mode of invasion and lymphocytic host response with known clinicopathological prognostic factors in oral squamous cell carcinoma

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## Abstract

**Background:** Oral squamous cell carcinoma (OSCC) is a significant public health problem in India, accounting to 30% of all cancers with a worrying rise in incidence and related mortality. Invasive tumor front (ITF) of OSCC has been an area of histopathologic research interest, where parameters like tumor budding (TB), mode of invasion (MOI) and lymphocytic host response (LHR) are being evaluated extensively.

**Objectives:** The aim is to study and evaluate the possible association of ITF histological parameters such as TB, LHR and MOI with known clinicopathological prognostic factors in cases of OSCC.

**Subjects and Methods:** We reviewed and analyzed 69 cases of OSCC for routine clinicopathological parameters, TB, MOI and LHR for any significant correlation ( $P < 0.05$  by Chi-square test) with each other and with outcome in cases where follow-up was available.

**Results:** TB correlated significantly with histological grade, worst pattern of invasion (WPOI), Lymphnodal involvement (LNI), Lymphovascular invasion (LVI), Perineural invasion (PNI) and age; MOI correlated with WPOI, LNI, LVI and PNI; and LHR significantly correlated with WPOI, PNI, Tumor size (pT) and outcome. TB showed a strong correlation with MOI ( $P < 0.001$ ) and LHR; and no significant association was noted between LHR and MOI. Among all the clinicopathological parameters, depth of invasion, pT, WPOI, PNI and LHR showed significant correlation with outcome.

**Conclusion:** TB, MOI and LHR showed good correlation with established parameters and as they are easy and helps in prognostication, they should be included in routine histopathological reporting guidelines.

**Keywords:** Invasive tumor front, lymphocytic host response, mode of invasion, oral squamous cell carcinoma, tumor budding

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## INTRODUCTION

In India, oral squamous cell carcinoma (OSCC) is a significant public health problem amounting to 30% of all cancers with worrying rise in incidence among both sexes.<sup>[1]</sup>

Cancer cells located in the tumor-host interface, also known as invasive tumor front (ITF), has been an area of histopathologic research interest, where parameters like tumor budding (TB), lymphocytic host response (LHR) and mode of invasion (MOI) are being evaluated extensively.<sup>[2]</sup>

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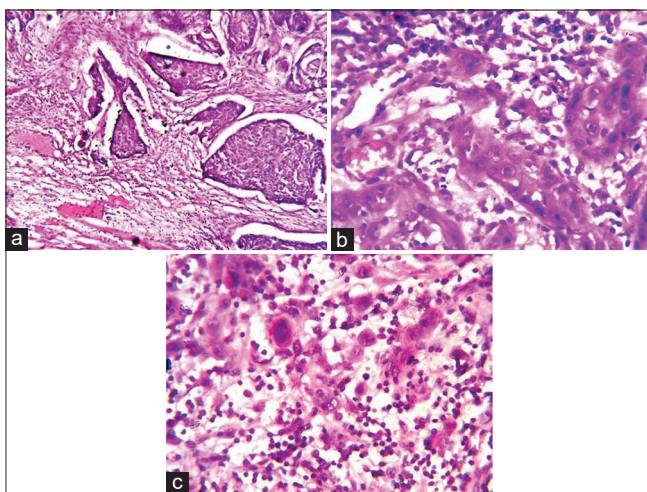
This study was conducted to evaluate the possible association of TB, LHR and MOI with known clinicopathological prognostic factors in cases of OSCC.

## SUBJECTS AND METHODS

With the approval of the Institutional ethical board, we conducted a study of all the cases of oral cavity Squamous cell carcinoma who underwent resection with cervical lymph node dissection in our institute from January 2018 to September 2019. Inclusion criteria were primary resection and concomitant neck dissection, and the availability of all the histopathologic slides of the resected specimens. Data such as age, sex, site and size of tumor (pT) were retrieved from archives, histopathological data like histological grade, worst pattern of invasion (WPOI), perineural invasion (PNI), lymphovascular invasion (LVI) and lymphnodal involvement (LNI) were reviewed and TB, MOI and LHR were evaluated on hematoxylin and eosin slides. Cases with prior radiotherapy were excluded from the study.

TB [Figure 1] is the presence of isolated cell or clusters of <5 cells at the ITF. The slides were initially viewed at  $\times 4$  objective lens to select areas with the highest density budding and the number of tumor buds in a hotspot field ( $0.78 \text{ mm}^2$ ) were counted using  $\times 40$  objective after making corrective calculations. The intensity of TB was categorized as low ( $<5/0.78 \text{ mm}^2$ ), intermediate intensity ( $5 \text{ to } <10/0.78 \text{ mm}^2$ ) and high intensity ( $\geq 10 \text{ buds}/0.78 \text{ mm}^2$ ) according to ITBCCC 2016 recommendations.<sup>[3]</sup>

MOI [Figure 2] was assessed on H- and E-stained sections, according to Yamamoto *et al.*<sup>[4]</sup> and graded from 1 to 4d. Tumors with Grade 1 MOI have well-defined borders, Grade



**Figure 1:** Tumor Budding, (a) Low intensity (H and E,  $\times 100$ ); (b) Intermediate intensity (H and E,  $\times 400$ ); (c) High intensity (H and E,  $\times 400$ )

2 tumors have cords and less well-defined borders. Grade 3 tumors exhibit groups of cells but no distinct borders. Diffuse cord-like invasion of tumors is graded as 4c while the tumors with single or fewer invasive cells are graded as 4d.

LHR [Figure 3] was graded semi-quantitatively as mild, moderate and heavy. The presence of band-like lymphocytic infiltrates with or without lymphoid aggregate was classified as heavy LHR, while the presence of only a few lymphocytes was graded as mild LHR. Lymphocytic infiltrate in between these two groups was graded as moderate LHR.<sup>[1,5]</sup>

All cases were further analyzed; patients with available follow-up data were evaluated for any significant relationship between the clinicopathological parameters, TB, MOI, LHR and overall survival. Follow-up till March 2019 (minimum 6 months) was obtained. All the deaths were due to metastasis or death in terminal stages of malignancy. No deaths noted due to comorbidities.

Chi-square test was used to analyze the correlation between TB, MOI, LHR and clinic-pathological parameters and also individually each parameter with overall survival. In the univariate analyses,  $P < 0.05$  was considered statistically significant.

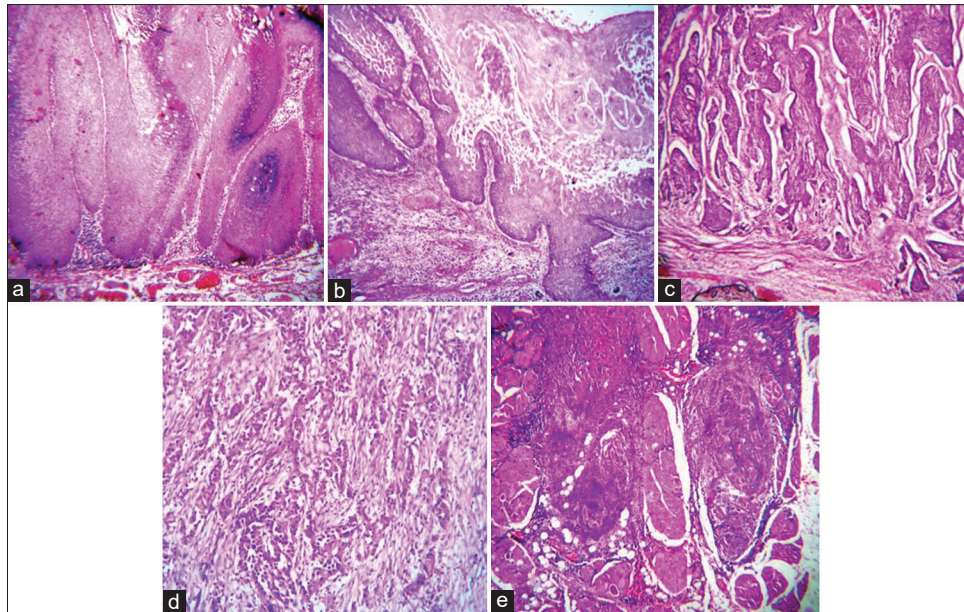
## RESULTS

According to the inclusion criteria, we collected the required data in 69 cases of OSCC and the slides were reviewed and evaluated for histopathological parameters. The age ranged between 25 and 87 years with a mean of 49.9 years. There were 56 male patients and 13 female patients with male-to-female ratio of 4.3:1. The most common site of OSCC was buccal mucosa (49%), followed by the tongue (43%), among which lateral border of tongue was the common site. The least common site was the maxilla (1.44%).

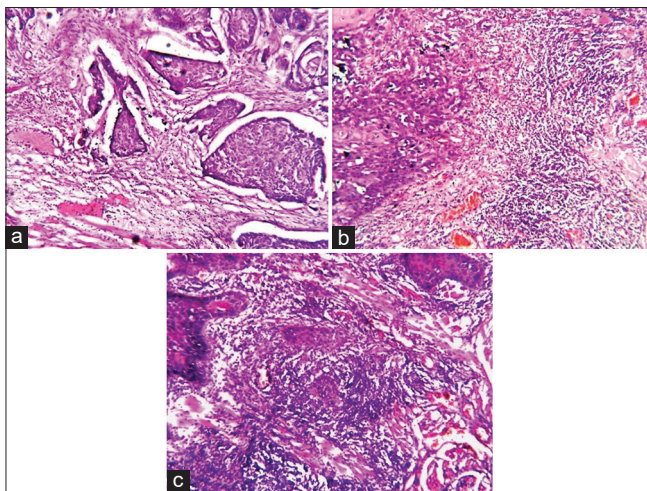
Among the 69 cases, 57 cases (82.6%) were grade 1 tumors, 9 cases (13%) were grade 2 and only 3 cases (4.3%) were grade 3 tumors. 63 cases (91.3%) showed  $>4 \text{ mm}$  depth of invasion (DOI) and 6 cases (8.6%) had  $<4 \text{ mm}$  DOI. 30 cases (43.4%) were pT2 tumors, 22 (31.8%) were pT3, pT1 were 11 (15.9%) and pT4 tumors were 6 (8.6%) in number. LNI was noted in 27 cases (39.2%). 0–4 WPOI score was seen in 48 cases (69.5%) and 21 cases (30.5%) had score of 5. LVI was seen in 45 cases (65.2%) and PNI in 32 cases (46.3%).

### Tumor budding [Table 1]

Low-intensity TB was seen in 42 cases (60.9%) followed by high-intensity TB in 17 cases (24.6%) and intermediate in 10 cases (14.5%). TB correlated significantly ( $P < 0.05$ )



**Figure 2:** Mode of invasion, (a) Grade 1 (H and E, ×100); (b) Grade 2 (H and E, ×100); (c) Grade 3 (H and E, ×100); (d) Grade 4c (H and E, ×100); (e) Grade 4d (H and E, ×100)



**Figure 3:** Lymphocytic host response: (a) Low intensity (H and E, ×100); (b) Intermediate intensity (H and E, ×400); (c) High intensity (H and E, ×400)

with histological grade, WPOI, LNI, LVI, PNI and age, while no significant correlation was noted with DOI, sex, site and pT, Table 1.

In the present study, majority of tumors were grade one (82.6%) tumors and low intensity of TB was the most common type noted (66.7%) among them. There were only 3 cases of grade 3 OSCC and all cases showed high-intensity TB. WPOI was divided according to CAP Protocol into 0–4 and 5 scores, majority of tumors showed 0–4 score (48 cases, 69.5%), among these low-intensity TB was the most common type (39/48, 81.3%). WPOI with 5 score was seen in 21 cases and high-intensity TB was the most common type among them (15/21, 71.4%).

In this study, a majority of 42 cases were node-negative cases and low intensity of TB was the most common type (31/42, 73.8%).

#### Mode of invasion [Table 2]

MOI was graded as 1 (2 cases, 2.9%), 2 (2 cases, 2.9%), 3 (31 cases, 44.9%), 4c and 4d (17 cases each, 24.6% each). There was a significant correlation between MOI and WPOI, LNI, LVI, PNI while no correlation was observed with histologic grade, DOI, pT, sex, site and age [Table 2].

In the current study, pattern 3 was the most common pattern (31 cases). There were 48 cases with WPOI 0–4 score, a majority of 30/48 (62.5%) showed MOI 3 and none of the 0–4 score case showed MOI 4d type. Among the 21 cases of WPOI 5 score, a majority of 17 cases showed MOI 4d type (80.9%).

Among the 42 cases with no nodal involvement, MOI 3 (24/42 57.1%) was the most common pattern. Among the node-positive cases type 4c was the most common MOI followed by type 4d and 3. Among the 32 cases of OSCC with PNI a majority of 15 cases (46.8%) showed MOI 4d followed by type 4c and type 3 MOI. Among the 45 OSCC cases with LVI, 4d and 4c type of MOI were the most common type (16/45, 35.5%). MOI type 3 was the most common type among the OSCC cases with no LVI (18/24, 75%).

#### Lymphocytic host response [Table 3]

Among the 69 cases, 46 cases (66.7%) showed moderate LHR, followed by 13 cases (18.8%) of mild LHR

**Table 1: Comparison of different clinicopathological parameters, mode of invasion and lymphocytic host response with tumor budding**

Parameter	Tumor budding (total number of cases 69)			P
	Low (n=42)	Intermediate (n=10)	High (n=17)	
Age (years)				
20- 29	1	2	0	0.031*
30- 39	6	2	8	
40- 49	12	3	4	
50- 59	11	1	2	
60- 69	7	1	1	
70- 79	5	0	2	
>80	0	1	0	
Sex				
Female	11	1	1	0.145
Male	31	9	16	
Grade				
1	38	8	11	0.024*
2	4	2	3	
3	0	0	3	
DOI				
<4	5	1	0	0.335
>4	37	9	17	
Pt				
1	8	2	1	0.155
2	22	4	4	
3	10	3	9	
4a	2	1	3	
LNI				
No	31	5	6	0.017*
Yes	11	5	11	
WPOI				
0- 4	39	7	2	0.000*
5	3	3	15	
LVI				
No	23	0	1	0.000*
Yes	19	10	16	
PNI				
No	31	4	2	0.000*
Yes	11	6	15	
MOI				
1	2	0	0	0.000*
2	2	0	0	
3	30	1	0	
4a	7	7	3	
4c	1	2	14	
LHR				
Mild	4	1	8	0.009*
Moderate	30	7	9	
Severe	8	2	0	

\*P<0.05 - Significant correlation. DOI: Depth of invasion, pT: Tumor size, LNI: Lymphnodal involvement, WPOI: Worst pattern of invasion, LVI: Lymphovascular invasion, PNI: Perineural invasion, MOI: Mode of invasion, LHR: Lymphocytic host response

and 10 cases (14.5%) of severe LHR. LHR showed significant correlation with WPOI, PNI and pT, Table 3.

Among the 69 cases, a majority of 48 cases had WPOI 0–4 score, moderate LHR was more common (35/48, 72.9%) among them. Among the 30 cases of OSCC with pT2, intermediate LHR was the commonest (25/30, 83.3%).

**Table 2: Comparison of different clinicopathological parameters and lymphocytic host response with mode of invasion.**

Parameter	Mode of Invasion (total number of cases 69)					P
	1 (n=2)	2 (n=2)	3 (n=31)	4c (n=17)	4d (n=17)	
Age (years)						
20- 29	0	0	1	2	0	0.817
30- 39	0	0	6	3	7	
40- 49	0	1	10	4	4	
50- 59	1	1	5	4	3	
60- 69	1	0	5	2	1	
70- 79	0	0	4	1	2	
>80	0	0	0	1	0	
Sex						
Female	0	1	7	4	1	0.389
Male	2	1	24	13	16	
Grade						
1	2	2	29	12	12	0.051
2	0	0	2	5	2	
3	0	0	0	0	3	
DOI						
<4	1	0	3	2	0	0.175
>4	1	2	28	15	17	
pT						
1	1	0	6	3	1	0.07
2	1	1	17	6	5	
3	0	0	7	8	7	
4a	0	1	1	0	4	
LNI						
No	2	2	24	6	8	0.016*
Yes	0	0	7	11	9	
WPOI						
0- 4	2	2	30	14	0	0.000*
5	0	0	1	3	17	
LVI						
No	2	2	18	1	1	0.000*
Yes	0	0	13	16	16	
PNI						
No	2	2	27	7	2	0.000*
Yes	0	0	7	10	15	
LHR						
Mild	0	0	2	4	7	0.057
Moderate	1	1	24	10	10	
Severe	1	1	5	3	0	

\* P<0.05 - Significant correlation. DOI: Depth of invasion, pT: Tumor size, LNI: Lymphnodal involvement, WPOI: Worst pattern of invasion, LVI: Lymphovascular invasion, PNI: Perineural invasion, LHR: Lymphocytic host response

There were 6 cases of pT4 stage OSCC and a majority of 3 cases showed mild LHR.

TB showed significant correlation with MOI and LHR, Table 1. No significant association was noted between LHR and MOI in the present study, Table 2.

In the present study, out of 69 cases, 33 cases follow-up was obtained with a minimum of 6 months follow-up. Out of 33, 20 patients were event-free and death was reported in 13 cases. PT, DOI, PNI, WPOI and LHR showed a statistically significant correlation (P < 0.05) with patient outcome, Table 4 (in discussion).

**Table 3: Comparison of different clinicopathological parameters with lymphocytic host response**

Parameter	Lymphocytic host response (total number of cases 69)			P
	Low (n=13)	Intermediate (n=46)	High (n=10)	
Age (years)				
20- 29	1	0	2	0.16
30- 39	3	12	1	
40- 49	4	11	4	
50- 59	0	13	1	
60- 69	3	5	1	
70- 79	2	4	1	
>80	0	1	0	
Sex				
Female	0	10	3	0.13
Male	13	36	7	
Grade				
1	9	40	8	0.23
2	2	5	2	
3	2	1	0	
DOI				
<4	1	3	2	0.387
>4	12	43	8	
Pt				
1	2	7	2	0.032*
2	1	25	4	
3	7	12	3	
4a	3	2	1	
LNI				
No	5	31	6	0.16
Yes	8	15	4	
WPOI				
0- 4	3	35	10	0.000*
5	10	11	0	
LVI				
No	2	17	5	0.19
Yes	11	29	5	
PNI				
No	3	26	8	0.02*
Yes	10	20	2	

\*P<0.05 - Significant correlation. DOI: Depth of invasion, pT: Tumor size, LNI: Lymphnodal involvement, WPOI: Worst pattern of invasion, LVI: Lymphovascular invasion, PNI: Perineural invasion

## DISCUSSION

Most of the cases of squamous cell carcinomas have a poor prognosis. TNM staging and LNI are the long known factors influencing the prognosis and outcome. If it is possible to elucidate potential clinicopathological parameters or predictors of nodal metastasis, they could be used to identify patients for the management of neck nodes. Many studies have evaluated various histological parameters that can predict cervical lymphnode metastasis, of which TB, LHR and pattern of invasion are some of the important factors.<sup>[2,5,6]</sup>

TB represents two malignant features: Cellular dyscohesion and active invasion.<sup>[7]</sup> IT is found to be associated with a higher incidence of nodal metastasis, recurrence and poor overall survival.<sup>[8]</sup> However, the pattern of tumor invasion/MOI and budding are

**Table 4: Comparison of inter-relationship between clinicopathological features, tumor budding, mode of invasion and lymphocytic host response with outcome in the present study and Chatterjee et al.<sup>[1]</sup> study**

Parameter	Present study (n=33)			Chatterjee et al. <sup>[1]</sup> (n=48)
	Alive (n=20)	Death (n=13)	P	
Sex				
Female	5	2	0.51	0.34
Male	15	11		
Grade				
1	14	12	0.051	0.73
2	6	0		
3	0	1		
DOI				
<4	9	0	0.005*	0.33
>4	11	13		
Pt				
1	5	0	0.049*	0.96
2	10	4		
3	4	6		
4a	1	3		
LNI				
No	12	5	0.22	-
Yes	8	8		
WPOI				
0- 4	17	6	0.01*	0.47
5	3	7		
LVI				
No	8	3	0.31	0.30
Yes	12	10		
PNI				
No	15	4	0.01*	0.24
Yes	5	9		
TB				
Low	14	9	0.73	0.44
Intermediate	3	1		
High	3	3		
MOI				
2	1	0	0.10	-
3	11	4		
4a	6	3		
4c	2	6		
LHR				
Mild	1	6	0.007*	0.62
Moderate	14	7		
Severe	5	0		

\*P<0.05 - Significant correlation. DOI: Depth of invasion, pT: Tumor size, LNI: Lymphnodal involvement, WPOI: Worst pattern of invasion, LVI: Lymphovascular invasion, PNI: Perineural invasion, TB: Tumor budding, MOI: Mode of invasion, LHR: Lymphocytic host response

not well-established prognostic factors and are not mentioned as required criteria for reporting of oral cavity malignancies according to the College of American Pathologist guideline. This is because of scarcity of literature available on ITF.<sup>[8]</sup>

LHR is an integral part of histopathologic risk score (RS) system of OSCC which is composed of three variables that reflect tumor–host inter-relationships: WPOI and PNI being the other two factors. This RS system has been shown to be significantly predictive of recurrence and overall survival.<sup>[9]</sup>

In the current retrospective study of 69 cases of OSCC, the mean age of presentation was 49.9 years with a range of 25–87 years. In contrast, studies done by Siriwardena *et al.*,<sup>[10]</sup> Watanabe *et al.*<sup>[11]</sup> and Manjula *et al.*<sup>[12]</sup> reported a higher mean of 57.8, 60.7 and 62.5 years, respectively. There was a male preponderance of 4.3:1, which is similar to the reports presented by these studies.<sup>[11,12]</sup>

### Tumor budding

TB is an inexpensive and easy to evaluate histological parameter that shows good interobserver agreement.<sup>[1]</sup> It has been suggested that cancer cells located in the ITF are more aggressive in terms of metastatic potential.<sup>[7]</sup> TB has been related to the prognosis of patients in various types of epithelial cancers, such as esophageal, lung, colorectal and endometrial carcinomas.<sup>[6]</sup>

TB was significantly associated with the overall survival of the OSCC patients and proposed to be an independent prognostic indicator by studies done by Boxberg *et al.*<sup>[13]</sup> and Pedersen *et al.*<sup>[14]</sup>

In the current study, TB showed significant correlation with histological grade, WPOI, LNI, LVI and PNI. No correlation was noted between TB and age, sex, site, DOI and pT. Wang *et al.*<sup>[7]</sup> reported similar significant association with grade and LNI. In contrast to this study, Wang *et al.*<sup>[7]</sup> also reported a significant correlation with pT.

Almangush *et al.*,<sup>[6]</sup> Wang *et al.*,<sup>[7]</sup> Angadi *et al.*<sup>[15]</sup> on OSCC, Seki *et al.*<sup>[16]</sup> on tongue SCC, have reported a significant relationship between TB and clinicopathologic factors such as lymph node involvement, vascular invasion, tumor stage and grade, DOI and the survival rate.

There were very few studies on the correlation of TB with WPOI. We noted a strong correlation between TB and WPOI (<0.001); in contrast, no correlation was noted in a study by Shimizu *et al.*<sup>[2]</sup>

Tumor budding was found to be a good predictor of clinically node-negative OSCC cases in some studies, further adding its utility as an important histopathological parameter.<sup>[8]</sup> Angadi *et al.*<sup>[15]</sup> reported high-intensity tumour budding to be a strong independent prognostic factor for the prediction of lymph node metastasis.<sup>[8]</sup>

Although some authors recommend to use different TB criteria for different subsites within the oral cavity, we evaluated them together, similar to Chatterjee *et al.*<sup>[1]</sup> because according to the current cancer staging system of the World Health Organization and American Joint Committee on Cancer, carcinoma of these sites are

staged using the same criteria and show similar biological behaviour.<sup>[1]</sup>

An important advantage of TB as an indicator of prognosis is its simplicity and the possibility to measure it repeatedly; In addition, it can be evaluated with conventional H and E staining with no need for expensive tools.<sup>[11]</sup>

### Mode of invasion

The pattern of invasion (MOI) at the advancing front of the tumor and level of differentiation are some of the individual histological parameters that help to predict regional lymph node metastasis.<sup>[17]</sup> Differentiated neoplastic cells have the tendency to invade the underlying connective tissue with pushing, well-delineated borders, the poorly differentiated cells of the tumor possess significantly infiltrative margins.<sup>[18]</sup> A significant correlation was found between the frequency of metastases and the type of invasive growth pattern.<sup>[19]</sup>

In the present study, MOI showed significant correlation with WPOI, LNI, LVI and PNI. Similarly, a significant association was reported by Khwaja *et al.*,<sup>[18]</sup> Seki *et al.*<sup>[16]</sup> and Siriwardena *et al.*<sup>[10]</sup> Shimizu *et al.*<sup>[2]</sup> also reported a significant relation between MOI and WPOI similar to the present study.

The pattern of tumor infiltration at the invasive front has been evaluated in a few previous studies. An invasive pattern of infiltration is associated with a higher risk of LN metastasis and poor disease-free survival.<sup>[20,6]</sup>

### Lymphocytic host response

LHR is assessed at the tumor interface light microscopically and demonstrated that strong LHR at the interface is associated with improved outcome, which is consistent with the concept that enhanced immune surveillance and adaptive immunity have a protective impact for cancer patients.<sup>[5]</sup>

In the present study, LHR showed significant correlation with WPOI, PNI and pT. No correlation was noted with LNI and was similar to studies done by Chatterjee *et al.*<sup>[1]</sup> and Manjula *et al.*<sup>[12]</sup>

Brandwein-Gensler *et al.*<sup>[21]</sup> in their study reported a correlation between LHR and WPOI, recurrence of tumor and overall survival. LHR showed no significant correlation with overall survival, disease recurrence and lymph node status in Batool *et al.*<sup>[22]</sup> study. Lundqvist *et al.*<sup>[23]</sup> also reported that correlation between intense inflammation and better response to radiotherapy and fewer recurrences.

### Interrelationship of tumor budding, mode of invasion and lymphocytic host response

There was a strong correlation ( $P < 0.001$ ) between TB and MOI indicating the higher intensity of TB in higher grades of MOI and vice versa, similar to results in Shimizu *et al.*<sup>[2]</sup> study. There was a significant correlation between TB and LHR, indicating good lymphocytic response in lower intensity of TB and mild lymphocytic response in higher intensity of TB. There was no correlation between LHR and MOI ( $P = 0.057$ ).

### Clinicopathological features in relation to outcome

Among all the clinicopathological parameters, in the present study, DOI, pT, WPOI, PNI and LHR showed significant correlation with outcome in contrast to Chatterjee *et al.*<sup>[1]</sup> study where no correlation was noted in any of the clinicopathological parameters studied. In contrast to the present study significant correlation was noted between TB and outcome in studies done by Wang *et al.*,<sup>[7]</sup> Seki *et al.*<sup>[18]</sup> (OSCC of all clinical stages), Almangush *et al.*,<sup>[6]</sup> and Attramadala *et al.*<sup>[24]</sup> (OSCC of clinical stage ½ only). All these studies had a larger sample size with longer duration of follow-up which is necessary to establish the significance of these parameters as predictors of survival and different cutoffs were used for TB. Hence, it still remains to be determined which evaluation methods for determining a budding score should be standardized among pathologists.<sup>[6]</sup>

### CONCLUSION

TB correlated significantly with histological grade, WPOI, LNI, LVI, PNI and age; MOI correlated with WPOI, LNI, LVI and PNI; and LHR significantly correlated with WPOI, PNI, pT and outcome. Parameters such as DOI, WPOI, PNI, pT and LHR help in predicting outcome. Based on the findings of this study and the previous studies, these parameters, TB, MOI, LHR may be used as novel diagnostic tools for planning management and should be routinely evaluated both in resection and in preoperative biopsy specimens and made a part of standard reporting format for OSCC as they are easy to assess, with minimal inter-observer variability and can be done on routine hematoxylin- and eosin-stained sections. These parameters if incorporated in routine reporting will help us in future with the prediction of clinical outcome and archiving of valued data for further analysis.

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### Conflicts of interest

There are no conflicts of interest.

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