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INVESTİGATİON OF TUMOR BUDDİNG İN ORAL SQUAMOUS CELL CARCİNOMA

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ABSTRACT

Background: Limited studies have been carried out on Tumor Budding in oral squamous cell carcinoma (OSCC). The present study evaluated the correlation between tumor budding and clinicopathologic factors in OSCC samples.
Materials and methods: Microscopic sections of 90 samples with definitive diagnosis of OSCC were evaluated. The clinicopathologic factors, were recorded and microscopic sections were evaluated the presence and scoring of tumor budding. Data were analyzed at a significance level of $P<0.05$.
Results: The 90 evaluated samples were consisted of 32 (36%) females and 58 (64%) males with age of 60 ±16 years old. The most common location was tongue as 38 cases (42.2%). 33% & 67% of investigated samples had low & high tumor budding, respectively. There was no significant correlation between tumor budding and patients' age and gender (P=0.54 & 0.88, respectively). Lymph node involvement, vascular invasion, grading and tumor size had significant correlation with tumor budding (P=0.0001). Also, there was a significant correlation between tumor budding and the depth of invasion (P=0.025). Based on Spearman's correlation coefficient, there was a positive correlation between all the factors evaluated in the present study except for age and gender.
Conclusion: The present study showed evaluation of tumor budding can be helpful to prediction of OSCC clinical behavior.

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Introduction

Oral squamous cell carcinoma (OSCC) comprises approximately 94% of all the malignancies of the oral cavity. In the United States, each year, almost 22000 new cases of OSCC were diagnosed and more than 5300 mortality cases were reported [1-5]. Early diagnosis of patients susceptible to the serious consequences of this tumor as invasion and progression is very important and should be considered to decrease aggressive treatment modalities which are associated with more severe complications.[6-8] There is now emphasis on histomorphometric parameters in some studies, which might be useful for predicting the behavior of tumors and their early diagnosis. Some of the known histomorphometric factors associated with malignancies include histology risk assessment score, tumor budding, depth of tumor invasion and the density of cancer-associated fibroblasts, of which tumor budding has attracted more attention due to the ease of its evaluation on conventional histopathologic sections.[9-11] Tumor budding is a manifestation of two characteristics related to tumors, including loss of cellular attachments and the aggressive behavior of the tumor, and is characterized by single cells separated from the tumor or clusters of cells that have been scattered at tumor margins in the anterior area of tumor invasion [12]. Microscopic findings in relation to tumor budding in some cancers have been introduced as a sign of poor prognosis and the aggressive behavior of the tumor and currently it is used as an indicator for the aggressive phenotype in colorectal cancers [13]. In addition, tumor budding has been reported as

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a useful clinical prognostic factor for esophageal SCC [14]. To the best of our knowledge, limited studies have been carried out to evaluate tumor budding in OSCCs and in the majority of these studies, lingual SCC has been specifically evaluated. Therefore, the present study was aimed to evaluate the correlation between tumor budding and clinicopathologic factors in OSCC samples.

Materials and Methods

In the present cross-sectional study, 90 samples with definitive diagnosis of OSCC (Oral Squamous cell Carcinoma) were selected from the archives of the Department of Oral & Maxillofacial Pathology, Dental Branch of Tehran, Islamic Azad University and Iran Cancer institute. The selected samples should have enough tissue with proper fixation procedure. The paraffin blocks of the samples were used to prepare 5-µm sections for H&E staining. First the clinicopathologic factors were evaluated, including grading, the presence of vascular invasion and the depth of invasion through measurement from the epithelial surface to the deepest area of tumor aggression, tumor size and involvement of the lymph node by assessing microscopic sections and the patient records. All microscopic parameters were evaluated by oral & Maxillofacial pathologist. In the next stage, the microscopic sections were evaluated using Olympus optic microscope in terms of the presence of tumor budding and its frequency was determined as follows: if separation of tumor cells from the main mass was observed individually or in aggregates with less than 5 cells at ×100 magnification, it was considered a focal spot for tumor budding. Then the samples were classified into two groups in relation to tumor budding: ≥3 as high and <3 as low.[12] Data we analyzed using chi-squared for correlation between tumor budding and sex, lymph node and vascular invasion, Kruskal-Wallis tests for correlation between tumor budding and grade and t-test at for this correlation with age; all considering significance level of $P<0.05$. Furthermore, spearman's correlation coefficient was calculated for the evaluation of correlation between tumor budding and clinicopathologic factors. Figures of 1-3 demonstrate presence and frequency of tumor budding in different grades of OSCC and figure 4 exhibits vascular invasion of this tumor.

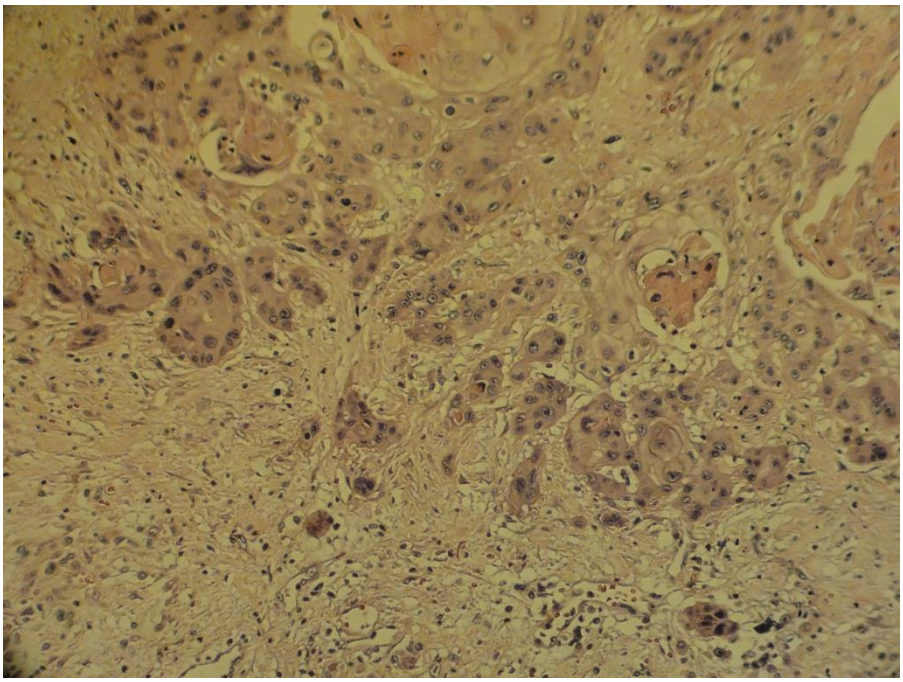

Figure 1. Microscopic sections show high tumor budding in well-differentiated OSCC at ×100.

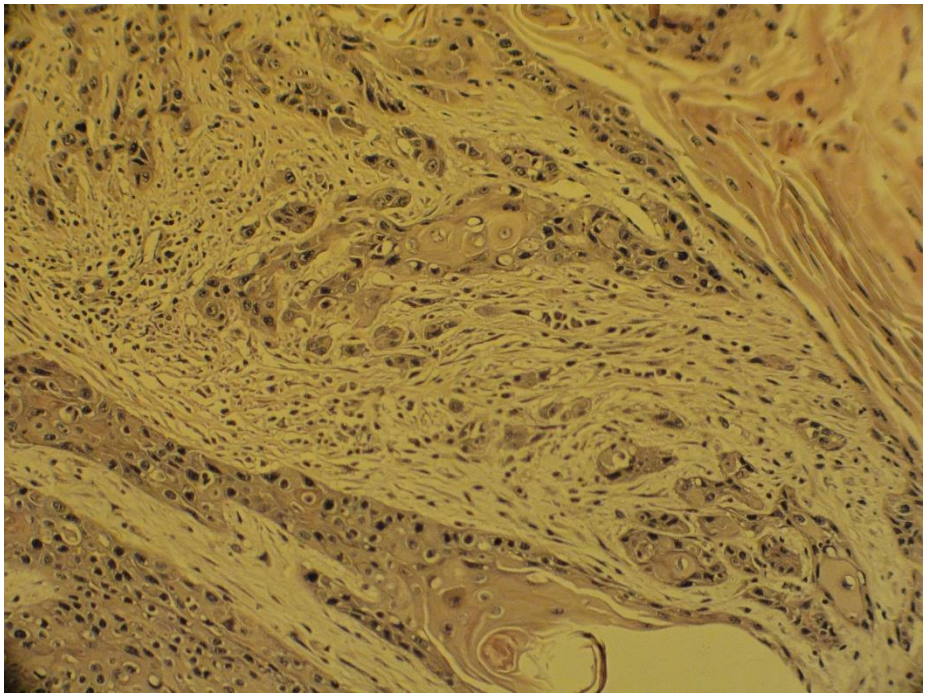

Figure 2. Microscopic sections show high tumor budding in moderately differentiated OSCC at

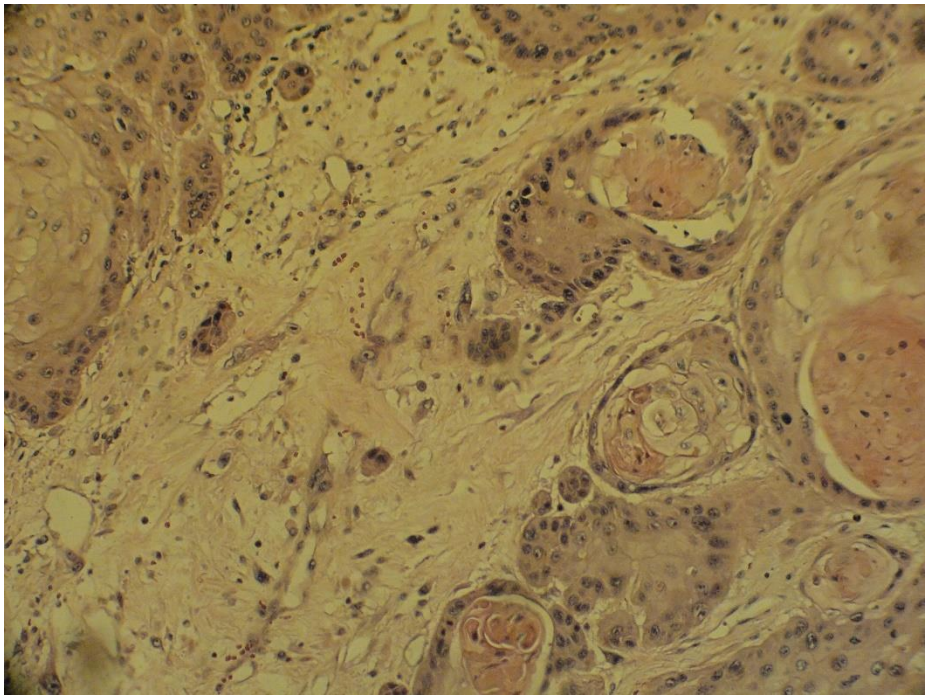

Figure 3. Microscopic sections showing low tumor budding in well-differentiated tumors at ×100.

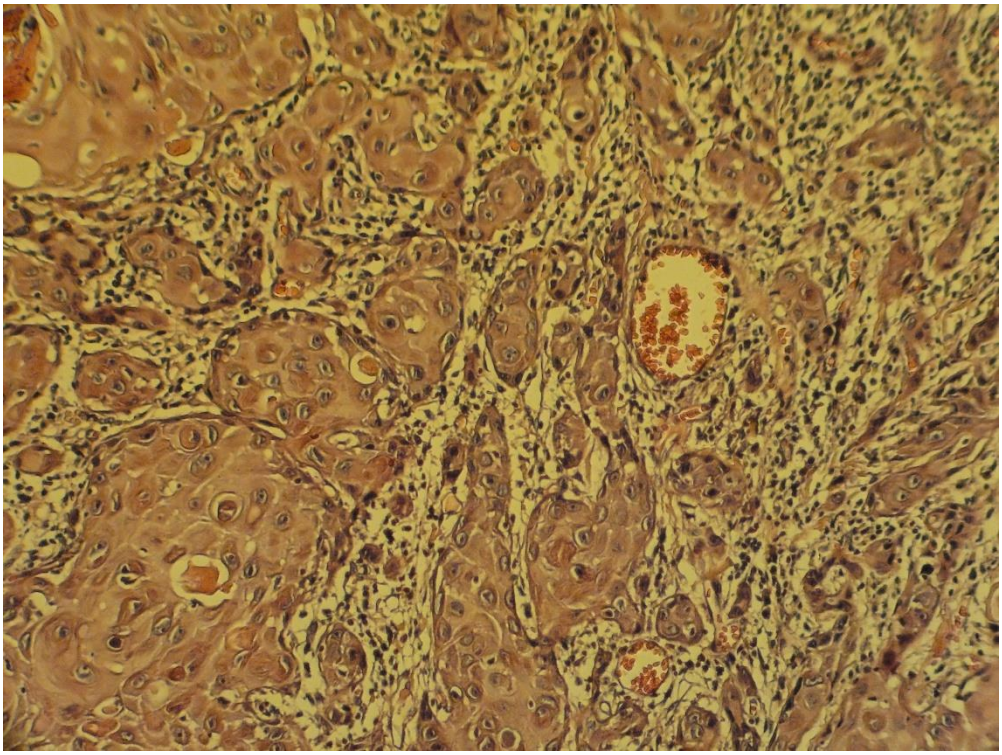

Figure 4. Microscopic section showing vascular invasion at ×100.

Results

Ninety samples evaluated consisted of 32 females (36%) and 58 males (64%), with a mean age of 60 years and standard deviation of 16 years and an age range of 22–88 years. In relation to tumor location, the tongue was the most common site with 38 cases (42.2%) and the other locations in descending order were as follows: 13 cases in the buccal mucosa (14.5%), 11 cases in the lower lip (12.2%), 10 cases in the palate (11.1%), 9 cases in the mandibular alveolar mucosa (10%), 5 cases in the retromolar pad (5.6%), 3 cases in the gingiva (3.3%) and one case in the upper lip (1,11%). In addition, 33% of the cases of OSCC evaluated exhibited low tumor budding, with 67% exhibiting high tumor budding.

(Table 1) presents the frequencies of tumor budding in terms of gender and clinicopathologic factors, including lymph node involvement, grade and vascular invasion.

Table 1. The frequencies of tumor budding statuses in terms of gender and clinicopathologic factors

		Tumor budding				P-value*
		low		**High**		
		Count	**%**	**Count**	**%**	
Gender	Female	10	33.3	22	36.7	0.818
	Male	20	66.7	38	63.3	
	Total	30	100.0	60	100.0	
Lymph node	-	27	90.0	25	41.7	0.0001
	+	3	10.0	35	58.3	
	Total	30	100.0	60	100.0	

Grade	Poor	0	.0	5	8.3	0.0001**
	Moderate	0	.0	29	48.3	
	Well	30	100.0	26	43.3	
	Total	30	100.0	60	100.0	
Invasion	-	23	76.7	7	11.7	0.0001
	+	7	23.3	53	88.3	
	Total	30	100.0	60	100.0	

*:calculated based on chi-squared test; **:calculated based on Kruskal-Wallis test

Based on the results of chi-squared test, there was no significant relationship between tumor budding and patients' gender (P=0.818); however, there was a significant relationship between lymph node involvement and vascular invasion and tumor budding (P=0.0001); in this context, 90% of subjects with low tumor budding (27 of 30) exhibited no lymph node involvement and 58% of subjects with high tumor budding (35 of 60) exhibited lymph node involvement. The same results were achieved in relation to vascular invasion, i.e. 76.7% of subjects with low tumor budding exhibited no vascular invasion and 53% with high tumor budding exhibited vascular invasion. In relation to the relationship between grading and tumor budding, Kruskal-Wallis test revealed a significant relationship between tumor budding and grading (P=0.0001); in this context, all the subjects with low tumor budding exhibited well-differentiated tumors and 91.7% of subjects with high tumor budding exhibited well-differentiated and moderately differentiated tumors.

(Table 2) presents, the statistics of tumor budding in terms of age and clinicopathologic factors, including tumor size and depth of invasion.

Table 2. The statistics of tumor budding in terms of age and clinicopathologic factors

		Tumor budding		**t statistics**	**P-value***
		Low	**High**		
Age	Mean	61.97	59.80	0.614	0.541
	SD	17.15	15.04		
	Minimum	22.00	24.00		
	Maximum	88.00	84.00		
	Mode	69.00	37.00		
Tumor size	Mean	3.76	5.09	-2.274	0.025
	SD	2.76	2.54		
	Minimum	1.00	1.00		
	Maximum	10.00	12.00		
	Mode	1.00	4.00		

Invasion depth	Mean	3.78	7.50	-7.583	0.0001
	S d	1.19	2.54		
	Minimum	1.50	.00		
	Maximum	5.50	12.00		
	Mode	4.50	8.00		

*: calculated based on independent samples t-test

Based on the results of t-test, tumor budding exhibited no significant relationship with patient age (P=0.54). However, there was a significant relationship between tumor size and invasion depth and tumor budding (P=0.025 and P=0.0001, respectively); in this context, in the group with high tumor budding, the tumor size and invasion depth were higher than those in the other group.

(Table 3) presents the correlation coefficients of tumor budding and clinico-pathological factors.

Table 3. The correlation coefficients between tumor budding and clinico-pathological factors

			Tumor budding
Spearman's rho	**Gender**	Correlation Coefficient	-.033
		p-value*	.759
		N	90
	Lymph node	Correlation Coefficient	.461
		p-value*	.0001
		N	90
	Invasion	Correlation Coefficient	.650
		p-value*	.0001
		N	90
	Grade	Correlation Coefficient	-.543
		p-value*	.0001
		N	90
	Age	Correlation Coefficient	-.081
		p-value*	.447
		N	90
	Tumor size	Correlation Coefficient	.288**
		p-value*	.006

		N	90
	Invasion depth	Correlation Coefficient	.709**
		p-value*	.0001
		N	90

*:calculated based on Spearman's Correlation Coefficient Test

Based on Spearman's correlation coefficient, there was a positive and significant correlation between tumor budding and all the factors except for age and gender ($P<0.05$). A positive correlation means subjects with high tumor budding had a tendency toward large tumor sizes, deeper invasion, higher grade (poorly differentiated), involvement of lymph nodes and vascular invasion.

Discussion

The results of the present study showed that tumor budding had a significant relationship with all the clinicopathologic factors evaluated, including lymph node involvement, grading, vascular invasion, tumor size and the depth of tumor invasion. In addition, based on Spearman's correlation coefficient, there was a positive and significant correlation between all the factors in this study and tumor budding except for age and gender.

To date, tumor budding has been evaluated in relation to the prognosis of esophageal, colorectal and skin cancers and some studies have associated it with poor prognosis of these tumors [15-21]. In the maxillofacial region, the number of studies in this respect is very limited and predominantly they have been related to tongue SCCs. In this context, studies by Almangush et al [12], Wang et al [22], Xie et al [23] Angadi et a [24] on SCC, a study by Seki et al [25] on tongue SCC and a study by Strieter et al [26] on labial SCC, have reported a significant relationship between tumor budding and clinicopathologic factors such as lymph node involvement, vascular invasion, tumor stage and grade, depth of invasion and the survival rate, consistent with the results of the present study. Based on previous studies, tumor budding is associated with a poor prognosis in patients with tongue, floor of the mouth and labial SCCs; in addition, a direct relationship has been reported between this factor and deep tumor invasion, repeated recurrence of disease and a decrease in patient survival. In addition, based on the results of studies above, tumor budding is a reproducible phenomenon and can be evaluated in future as an independent factor in SCC of the areas mentioned above.

The only study on OSCC that also evaluated other oral areas, is prepared by Sarioglu et a [27], in which there was no significant relationship between tumor budding and clinicopathologic factors such as tumor size, involvement of lymph nodes and the survival rate in 47 OSCC samples, contrary to the results of the present study. The discrepancy between the results of these two studies might be attributed to the small sample size in that study compared to the present study in which, 90 cases of OSCC were evaluated. In addition, in the present study, an attempt was made to evaluate the relationship between tumor budding and other clinicopathologic factors such as tumor size, depth of invasion, grading of the tumor and vascular invasion in addition to the factors above. It should be pointed out that due to the incomplete data in patient files it was not possible to evaluate tumor stage and patient survival rate. In addition, in the present study, the correlation between tumor budding and the clinicopathologic factors above was evaluated and based on the results, tumor budding exhibited a positive correlation with all the factors evaluated. Therefore, it can be concluded that tumor budding, in association with other clinicopathologic factors mentioned in this study, can be useful to determine the clinical behaviors of OSCC tumors.

An important advantage of tumor budding as an indicator of prognosis is its simplicity and the possibility to measure it repeatedly; In addition, it can be evaluated with conventional H&E staining with no need for expensive tools. Currently, tumor budding has been accepted and is used as an important histopathological parameter for the evaluation of malignancy grade and prognosis of large intestine cancers [16,17]. In addition, tumor budding in recognized as an invasion signal of cancer cells with poor prognosis in the early stages of large intestine cancers [18].

There are limited studies available on oral malignant conditions and there are doubts about the use of tumor budding as an indicator for the prediction of tumor behavior. In some cases there are problems in evaluation of clinicopathologic factors related to tumor clinical behavior such as the involvement of lymph nodes, vascular invasion, determination of stage and the survival rate; these problems include deficiencies in patient files, the patients' insufficient medical work-up and lack of patient compliance to return for follow-up evaluations. In this context, in appears use of parameters that, apart from having a correlation with the factors above, have advantages such as ease of evaluation and reproducibility, is considered one of the most important achievements in the evaluation of the clinical behavior of malignant tumors, resulting in proper treatment and a decrease in complications. Therefore, considering the results of the present study, in which there was a positive correlation in OSCC cases evaluated between tumor budding and clinicpathologic factors such as lymph node involvement, grading, tumor size, vascular invasion and invasion depth, it appears evacuation of tumor budding in OSCC can help predict tumor behavior. However, further studies are recommended to achieve further solid evidence in relation to the correlation mentioned. If future

studies confirm the results of the present study, it is hoped that this simple technique can be used to predict the behavior of OSCC lesions.

Conclusion

The results of the present study showed a correlation between tumor budding and clinicopathologic factors such as lymph node involvement, grading, tumor size, vascular invasion and the depth of invasion. Therefore, it seems tumor budding can help predict the behavior of OSCC lesions.

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