

Analysis of the invasive edge in primary and secondary oral squamous cell carcinoma: An independent prognostic marker: A retrospective study

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Abstract

Background and Objectives: Oral squamous cell carcinoma (OSCC) is one of the most common head and neck carcinomas and corresponds to 95% of all oral cancers with an increasing morbidity and mortality. Its prognosis is affected by several clinicopathologic factors, one of which is pattern of invasion (POI). The histological features of OSCC may differ widely, but there is general agreement that the most useful prognostic information can be deduced from the invasive front of the tumor. In this retrospective study, our aim was to compare the POI, the status of connective tissue and the status of inflammation at the tumor–host interface in primary and recurrent (secondary) OSCC and test the validity of POI, to serve as a potential marker to assess the prognosis of the patient.

Materials and Methods: Differentiation of tumors, POI, status of connective tissue and inflammation was assessed in 168 cases of primary and recurrent cases of OSCC.

Statistical Analysis: Fisher's exact test was used to determine the statistical significance and $P < 0.05$ was considered to be statistically significant.

Results: Our study showed that majority of the primary and secondary tumors were well differentiated, 117 [95.9%] and 34 [73.9%], respectively. Predominant POI in the primary and secondary tumor group was Pattern II and least was Pattern V. Worst pattern in primary tumor and highest distribution was seen for Pattern III (53.3%), and least for Pattern V (0.00%). In secondary tumors, the predominant worst pattern was Pattern IV (50.0%) and least distribution was seen for Pattern I (0.00%). Connective tissue status for both primary and secondary tumors showed the predominance of loose type (85.2% and 79.2%) and least was variable type (0.8% and 0.6%), respectively. Status of inflammation in the primary tumor group showed a predominance of moderate grade of inflammation (50.0%) and very mild grade of inflammation (6.6%) was the least type. In the secondary tumor group, moderate grade (43.5%) of inflammation was predominant and very mild grade (5.4%) was the least. All the parameters showed a statistically significant difference on the application of Fisher's exact test between the two groups.

Conclusion: Our study showed that POI could serve as an individual prognostic marker irrespective of the histologic differentiation of tumor. Tumor desmoplasia could be considered as an important reflection of the tumor-host interaction, especially in aggressive cancers. Host immune defense, especially tumor infiltrating

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lymphocytes must be noted as critical factors related to survival rate in OSCC patients. Assessment of mentioned parameters may lead to sound prognostic assessment and appropriate treatment planning thus reducing the possibility of recurrence or relapse. Hence, the parameters evaluated in our study could serve as independent or interdependent prognostic markers.

Key Words: Connective tissue status, inflammatory status, oral squamous cell carcinoma, pattern of invasion, primary and recurrent tumors

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is one of the most common head and neck carcinomas and corresponds to 95% of all oral cancers with an increasing morbidity and mortality.^[1] Despite substantial developments in both diagnosis and therapy in recent decades, the prognosis remains poor.^[2,3] Extensive local invasion and/or frequent regional lymph node metastases are usually present even at initial diagnosis, resulting in the unpredictable prognosis of OSCC.^[4] Clinical assessment by the tumor node metastasis (TNM) system suffers a major criticism that it ignores individual histological characteristics of tumors. Therefore, many workers have devised histological grading systems to predict the biological behavior and recommended prognostic markers for OSCC such as cell morphometry, proliferation-associated markers, flow cytometry and oncogene expression. Although many of these systems have prognostic significance in patients with OSCC, no prognostic predictors are reliable enough for clinicopathological use.^[5]

Multiparameter prognostic models and scoring systems that include nuclear pleomorphism, mitotic index, lymphocytic response, tumor growth pattern, tumor thickness, degree of keratinization, depth of invasion and pattern of invasion (POI) have been developed.^[6-9] The histological features of OSCC may differ widely, but there is general agreement that the most useful prognostic information can be deduced from the invasive front of the tumor, where the deepest and presumably most aggressive cells reside.^[5]

In this retrospective study, our aim was to compare the POI, the status of connective tissue and the status of inflammation at the tumor–host interface in primary and recurrent (secondary) OSCC and test the validity of POI, to serve as a potential marker for prognosis of the patient.

MATERIALS AND METHODS

Tissue specimens

One hundred and eighty paraffin-embedded sections were obtained from the cancer registry out of which 126 were primary OSCC and 54 were secondary tumors. Twelve cases, four from the primary tumor group and eight from secondary

tumor group were excluded from the study owing to inadequate representation of the deepest invasive front. Thus, a total of 168 cases of OSCC were included in the study. The study of all specimens received ethical approval.

Histopathological evaluation

All the hematoxylin and eosin stained histopathological slides were concurrently reviewed and evaluated independently by two qualified pathologists (the first observer and the second observer) using the same type of microscope without any prior knowledge of each patient's clinical details. A set criterion was formulated for evaluation of the slides and when the opinions of the two evaluators differed, consensus was reached by discussion.

Histologic variables and Invasion pattern grading

Tumor differentiation was done using Broder's grading system. Tumor POI was examined at the host/tumor interface. POI Type 1 through Type IV that had been previously defined by Bryne *et al.* was used for assessment. POI type V as defined by Brandwein *et al.* was also added. POI Type 1 represents tumor invasion in a broad pushing manner. POI Type 2 represents tumor invasion with broad pushing "fingers," or separate large tumor islands, with a stellate appearance. POI Type 3 represents invasive islands of tumor >15 cells per island. POI Type 4 represents invasive tumor islands smaller than 15 cells per island. This includes single cell invasion. POI Type 4 also includes strands of tumor cells in a single-cell filing pattern, regardless of island size. POI Type 5 represents tumor satellites of any size with 1 mm or greater distance of intervening normal tissue (not fibrosis) at the tumor/host interface [Figure 1a-e].^[10] All prior publications concerning POI were either limited to biopsy specimens or were based on the most aggressive POI present. To validate this practice, we used two new variables: POI-predominant existing pattern (POI-PEP) and POI-worst existing pattern (POI-WEP). POI-PEP was determined by measuring POI at the tumor interface of each slide. POI-PEP was tallied as the most common POI found; in case of a tie (e.g. four slides of POI-PEP 3 and four slides of POI-PEP 4), the higher score was assigned. The POI-WEP was taken as the highest score present, no matter how focal. All the slides were multisampled and the complete invasive edge was evaluated.

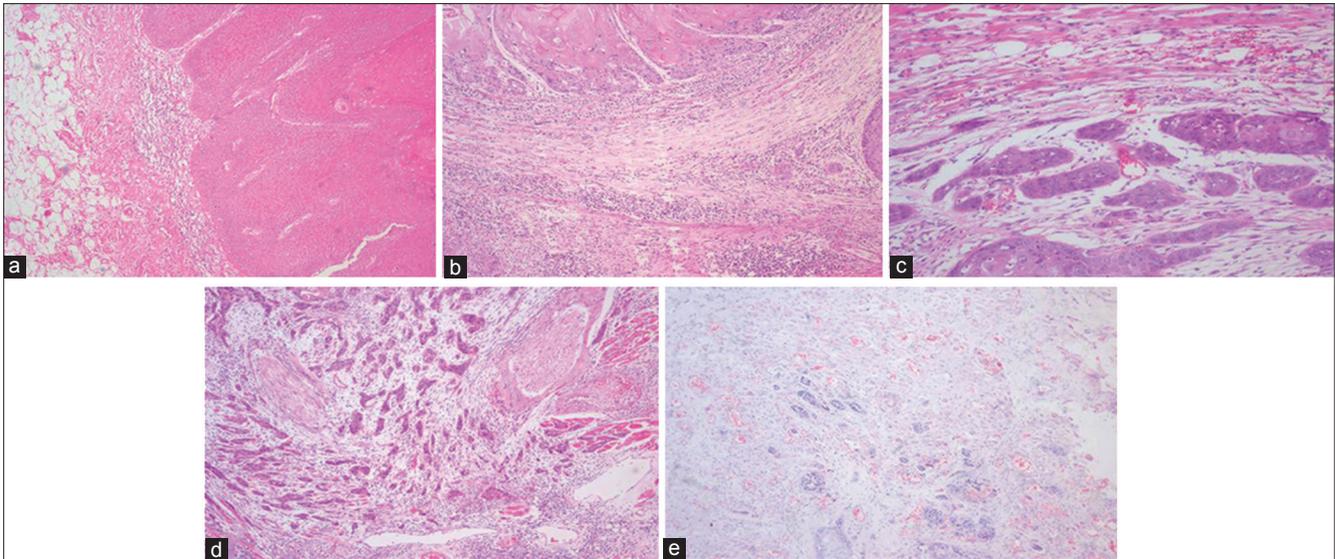


Figure 1: Photomicrograph showing various patterns of invasion. (a) Pattern of invasion-1, (b) Pattern of invasion-2 (c) Pattern of invasion-3 (d) Pattern of invasion-4 (e) Pattern of invasion-5 (H&E stain, x100)

Other variables examined were the status of connective tissue at the tumor–host interface between primary and secondary tumor and graded as loose, hyalinized, desmoplastic and variable type immediate to the invasive edge irrespective of the submucosa and the deeper plane. Most frequent pattern and any change in the pattern were noted.

The status of inflammation at the tumor–host interface between primary and secondary tumor was also examined and graded as very mild, mild, moderate and dense. Very mild grade was given when there was focal infiltration of inflammatory cells, and the background connective tissue was completely visible, mild grade when the inflammatory cells were less in number and the connective tissue was less visible as compared to very mild type, moderate when the number of cells increased and little stroma was seen. Dense grade was given when the number of cells increased tremendously forming aggregates, and the stroma was not visible.

Statistical analysis

All the data were tabulated, and statistical tests were performed using the Statistical Software Package (SPSS for windows 7). Fisher’s exact test was used to determine the statistical significance and $P < 0.05$ was considered to be statistically significant.

RESULTS

Comparison of primary and secondary tumor with differentiation [Table 1 and Figure 2]

Tumor differentiation pattern assessed using Broder’s system of grading showed that 95.9% of primary tumors were well differentiated, 3.3% moderately differentiated and 0.8% was poorly differentiated. About 73.9% were well differentiated,

Table 1: Comparison of primary and secondary tumor with differentiation

Differentiation	Primary (%)	Secondary (%)	Total (%)
Moderate	4 (3.3)	11 (23.9)	15 (8.9)
Poor	1 (0.8)	1 (2.2)	2 (1.2)
Well	117 (95.9)	34 (73.9)	151 (89.9)
Total	122	46	168
<i>P</i>		<0.001*	

Fisher’s exact test. * $P < 0.05$ statistically significant

23.9% were moderately differentiated and 2.2% were poorly differentiated in secondary tumor category.

When compared between primary and secondary tumors, highly statistical significant difference was observed between the two groups on application of Fisher’s exact test ($P = 0.001$).

Comparison of primary and secondary tumor with respect to status of squamous cell carcinoma predominant pattern [Table 2 and Figure 3]

The predominant POI in the primary tumor group was Pattern II (56.6%) followed by Pattern III (31.1%), Pattern I (11.5%) and the least pattern was Pattern V (0.0%). Same type of distribution was seen in the secondary tumor group with Pattern II (53.6%) predominating followed by Pattern III (33.9%), Pattern I (8.9%), Pattern IV (3.6%) and least pattern was Pattern V (6.5%). A highly statistical significant difference was observed between the two groups on the application of Fisher’s exact test ($P = 0.003^*$).

Comparison of primary and secondary tumor with respect to status of squamous cell carcinoma worst pattern [Table 3 and Figure 4]

When comparing the SCC worst pattern in the primary tumor, highest distribution was seen for Pattern III (53.3%)

and least for Pattern V (0.00%). In secondary tumors, Pattern IV (50.0%) predominated and least distribution was seen for Pattern I (0.00%). A high statistical significant difference was

observed between the two groups on application of Fisher's exact test ($P = 0.001$).

Table 2: Comparison of primary and secondary tumor with respect to status of squamous cell carcinoma predominant pattern

POI-PEP	Primary (%)	Secondary (%)	Total (%)
I	14 (11.5)	1 (2.2)	15 (8.9)
II	69 (56.6)	21 (45.7)	90 (53.6)
III	38 (31.1)	19 (41.3)	57 (33.9)
IV	1 (0.8)	5 (10.9)	6 (3.6)
V	0 (0)	0 (0)	0 (0)
Total	122	46	168
P		0.003*	

Fisher's exact test. * $P < 0.05$ statistically significant. POI: Pattern of invasion; POI-PEP: Predominant existing POI

Table 3: Comparison of primary and secondary tumor with respect to status of squamous cell carcinoma worst pattern

POI-WEP	Primary (%)	Secondary (%)	Total (%)
I	3 (2.5)	0	3 (1.8)
II	17 (13.9)	1 (2.2)	18 (10.7)
III	65 (53.3)	19 (41.3)	84 (50.0)
IV	37 (30.3)	23 (50.0)	60 (35.7)
V	0	3 (6.5)	3 (1.8)
Total	122	46	168
P		0.001*	

Fisher's exact test. * $P < 0.05$ statistically significant. POI: Pattern of invasion; POI-WEP: POI-worst existing pattern

Comparison of primary and secondary tumor with respect to status of connective tissue [Table 4 and Figure 5]

Connective tissue status for primary tumors showed the predominance of loose type (85.2%) and least was variable type (0.8%), and the same was true with the secondary tumor with loose type predominating (79.2%) and least being variable type (0.6%). On application of Fisher's exact test ($P = 0.002^*$), highly statistical significant difference was observed between the two groups.

Comparison of primary and secondary tumor with respect to status of inflammation [Table 5 and Figure 6]

Status of inflammation in the primary tumor group showed a predominance of moderate grade of inflammation (50.0%) and very mild grade inflammation (6.6%) was the least type. In secondary tumor group, moderate grade (43.5%) was predominant and very mild grade (5.4%) was the least.

When compared between primary and secondary tumors, a high statistical significant difference was observed between the two groups on application of Fisher's exact test ($P = 0.001^*$).

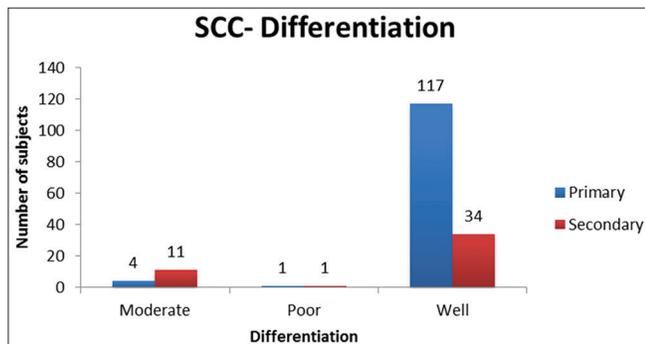


Figure 2: Graph depicting differentiation of primary and secondary oral squamous cell carcinoma

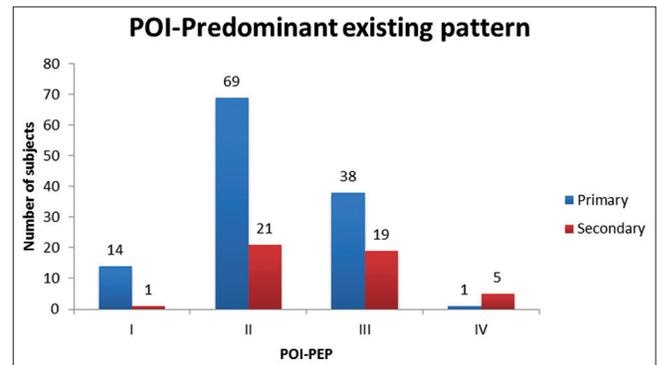


Figure 3: Graph depicting predominant pattern of invasion of primary and secondary oral squamous cell carcinoma

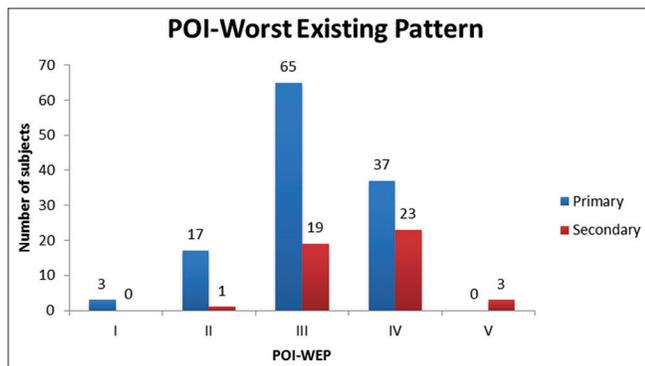


Figure 4: Graph depicting worst existing pattern of invasion of primary and secondary oral squamous cell carcinoma

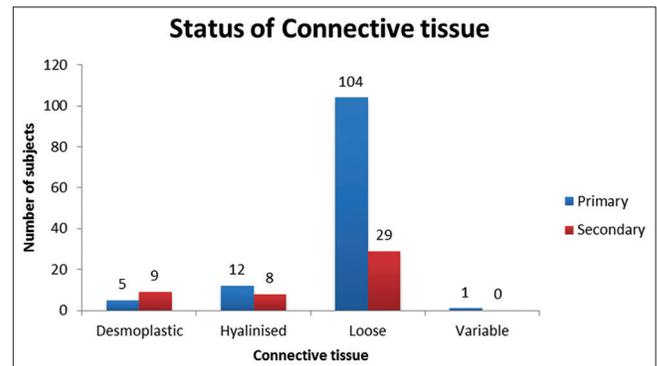


Figure 5: Graph depicting status of connective tissue at the tumor–host interface of primary and secondary oral squamous cell carcinoma

Table 4: Comparison of primary and secondary tumor with respect to status of connective tissue

Connective tissue	Primary (%)	Secondary (%)	Total (%)
Desmoplastic	5 (4.1)	9 (19.6)	14 (8.3)
Hyalinized	12 (9.8)	8 (17.4)	20 (11.9)
Loose	104 (85.2)	29 (63.0)	133 (79.2)
Variable	1 (0.8)	0.0	1 (0.6)
Total	122	46	168
P		0.002*	

Fisher’s exact test. *P<0.05 statistically significant

Table 5: Comparison of primary and secondary tumor with respect to status of inflammation

Inflammation	Primary (%)	Secondary (%)	Total (%)
Dense	15 (12.3)	2 (4.3)	17 (10.1)
Mild	37 (30.3)	32 (69.6)	69 (41.1)
Moderate	62 (50.8)	11 (23.9)	73 (43.5)
Very mild	8 (6.6)	1 (2.2)	9 (5.4)
Total	122	46	168
P		<0.001*	

Fisher’s exact test. *P<0.05 statistically significant

Most of the inflammatory response was chronic in nature and very rarely a mixed inflammatory response was seen.

DISCUSSION

Among the various aspects associated with cancer, factors affecting prognosis, probably remain the least understood and thereby an accurate prediction of outcome has been extremely challenging. Multiple factors have been implicated in the overall survival and recurrence of patients with head and neck cancer, the invasive tumor front being one of them. It refers to the manner in which cancer infiltrates tissue at the tumor/host interface and is patterned by multiple characters that specify degree of keratinization, lympho-plasmacytic infiltration, nuclear pleomorphism and pattern of tumor invasion (POI).^[10] The invasive tumor edge frequently shows a lower degree of differentiation and a higher grade of cellular dissociation in comparison with other parts of the tumor. It is believed that integral prognostic information about the tumor’s invasive and metastatic capacity can be deduced from it, where the deepest and presumably most aggressive cells reside.^[11-13] Over the past two decades, these features, especially POI, individually and as part of weighted scoring systems, have been demonstrated to predict local recurrence and overall survival. Several other studies have reported the prognostic significance of invasive front in OSCCs.^[7,10,11,14-16]

In addition to subjective histological interpretations of OSCC by pathologists, the major problem encountered when investigating OSCC is its heterogeneity both between tumors, within individual tumors and between close but biologically different oral anatomical sites. Thus, to avoid ambiguity, we used the Broder’s grading system, which is simple and easy to use. In our study, the majority of the cases were well

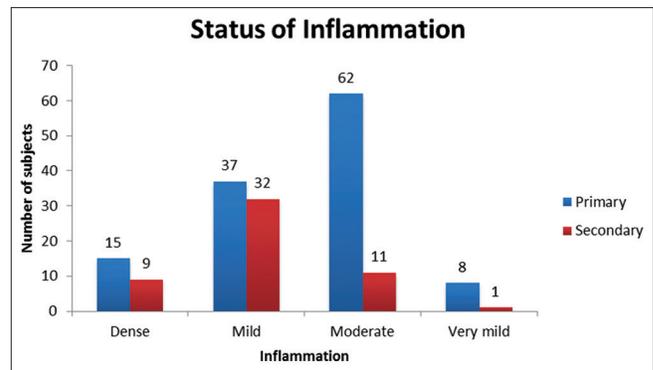


Figure 6: Graph depicting status of inflammation at the tumor–host interface of primary and secondary oral squamous cell carcinoma

differentiated both in primary tumor (95.9%) and secondary tumor (89.9%) group, and we observed that the differentiation of the tumors had no role to play in the POI status of both primary and secondary tumors.

Different POIs have been reported for squamous cell carcinomas of different sites, for example, the skin, tongue, head and neck, as well as the cervix uteri squamous cell carcinomas and, gastric and endometrial adenocarcinomas.^[17-22] A high grade of tumor cell dissociation, represented by dissociative, non-cohesive tumor growth at the front of the invasion, morphologically characterized by an infiltration of small tumor cell clusters into the surrounding tissue (i.e. spray-like pattern), has been reported to be of prognostic value in different types of carcinomas.^[23,24]

In SCC of the skin and the lower lip, a high grade of tumor cell dissociation, represented by a spray-like POI, was significantly associated with a high frequency of metastatic as well as recurrent disease.^[23-27] A reduced 5-year survival has been reported by Spiro *et al.* for patients with oral tongue cancer.^[20,23]

Our intention was to build upon previous studies regarding the predictive value of POI at the tumor interface. We used the Bryne *et al.* grading of POI with an addition of the POI V by Brandwein *et al.* Our study showed a POI-II to be the predominant POI in both primary (56.5%) and secondary tumors (45.7) of OSCC which was statistically significant, and the worst existing pattern was POI III (53.3%) for primary tumors and POI IV (6.5%) for secondary tumors. The POI V (6.5%) was seen only in the secondary tumor group. Thus, when compared between the tumor groups, a higher grade of WEP was seen in the secondary tumor group which could denote the aggressiveness of the secondary tumors. A clinicopathologic correlation and TNM status correlation could further help us to justify the significance of POI.

Malignancy is a state that emerges from a tumor–host microenvironment in which malignant tumor cells recruit

vasculature and stroma through the production and secretion of growth factors and chemokines. The locally activated host microenvironment (cellular and extracellular matrix) controls the proliferative and the behavior of the tumor cells. It also creates a permissive field to supply nutrients by angiogenesis and provides a pathway for metastasis through the vascular system.^[28]

Cancer cells not only destroy the preexisting extracellular matrix but also cancer invasion *per se* usually induces new matrix formation by activating the peritumoral stromal cells, that initiates desmoplastic stromal reaction (DSR).^[29] The DSR at the front of invasion (juxta-tumoral stroma) contains proliferating myofibroblasts, inflammatory cells, trapped residual atrophic parenchymal components of the invaded organ and also the process of neovascularization.^[24,29,30]

In our study, the loose type of connective tissue response was seen at the tumor–host interface in both primary and secondary tumor groups and when compared the DSR was higher in the secondary tumor group (19.6%) followed by the hyalinized type (17.4%). This could be one hallmark of the morphologic diagnosis of an invasive tumor and may be the result of a complex cross-talk between the tumor cells and the surrounding tissue.

Low tumor differentiation was significantly correlated with the spray-like POI and a moderate or strong DSR. This phenomenon was seen in squamous cell carcinomas of the skin, oral tongue,^[20,25] and the uterine cervix indicating that poorly differentiated tumors may induce a strong remodeling process in the juxtatumoral stroma.^[24]

Cancer-associated inflammation is a double-edged sword. Most of the components of cancer-promoting inflammation have a dual role in tumor development. They can either function as a pro- or anti-tumorigenic molecules and factors based on their expression levels, abundance, duration and state of activation in the tumor microenvironment.^[31]

The most common lymphocytic response in the primary tumor group was moderate type (30.3%), and in secondary tumor group, mild type predominated (69.6%). Various studies have demonstrated an inverse relationship between lymphocytic infiltrate and potential for lymph node metastasis^[6] as well as survival.^[32] Hosal *et al.* have demonstrated that poor lymphocyte response was associated with loco-regional recurrence.^[10,33] This was also in accordance with our study.

To summarize, it is well documented that the deepest and presumably most aggressive cells reside at the invasive front of tumors, and these show lesser degree of differentiation. The

POI could serve as an individual prognostic marker and tumor desmoplasia, could be considered as an important reflection of the tumor–host interaction, especially in aggressive cancers. Host immune defense, especially tumor infiltrating lymphocytes must be noted as critical factors related to survival rate in OSCC patients. Assessment of mentioned parameters may lead to sound prognostic assessment and appropriate treatment planning thus reducing the possibility of recurrence or relapse. Hence, the parameters evaluated in our study could serve as independent or interdependent prognostic markers.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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