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Original Research

Inflammation And Connective Tissue At Invasive Tumor Front-A Reliable Parameter

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ABSTRACT:
Of the many oral malignancies, 95% attributes to OSCC and invasion and metastasis is responsible mainly for morbidity and mortality. Inflammation and connective tissue has a specific role in disease invasion and progression. The aim of this study was to check the correlation between status of inflammation and status of connective tissue at invasive tumor front with respect to oral squamous cell carcinoma cases. Both primary and secondary oral squamous cell carcinoma cases were evaluated. We have found significant correlation between these parameters and broders grading system at invasive tumor front.

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INTRODUCTION

Cancer cells at the tumor-host interface, the ITF, have been an area of interest for histopathological research nowadays. Various parameters like tumor budding, Pattern of invasion, Depth of invasion, Mode of invasion, Lymphocytic host response, Status of connective tissue, etc. have been studied extensively. Lymphocytic host response and status of connective tissue have now become an integral part of scoring system of oral squamous cell carcinoma cases. These parameters have shown to be significantly predictive of recurrence and overall survival.(1) The pattern of tumor invasion at ITF has been studied in few previous studies. In the present study we have evaluated status of inflammation and status of connective tissue at ITF in both primary and secondary OSCC cases. Previous studies have shown correlation between intense inflammation and better response to radiotherapy and chemotherapy with fewer recurrences. In the present study, we have tried to find out correlation between status of inflammation and status of connective tissue at ITF.

MATERIAL AND METHODS

The study was conducted in Department of Oral and Maxillofacial Pathology at Karnavati school of Dentistry during the period of 2016-19. A total of 200 clinically diagnosed and histologically confirmed Oral Squamous Cell Carcinoma cases were collected from The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad during the time period of 2016 to 2019. Out of which 150 cases were of primary OSCC and 50 cases were of secondary OSCC. Written consent was obtained and required patient data was collected. Routine hematological investigations were carried out for all patients. Tissue specimens were routinely fixed, processed and stained by Haematoxylin & Eosin stain. Slides were multisampled from tissue samples of all the cases for complete evaluation of tumor invasive front. Tumor differentiation was done

using Broder's grading system (1920) as well differentiated OSCC, moderately differentiated OSCC and poorly differentiated OSCC cases. Status of connective tissue at the tumor–host interface in primary and secondary tumor was evaluated immediate to the invasive front as: Loose, Hyalinized, Desmoplastic, Variable. Status of inflammation at the tumor–host interface in primary and secondary tumor was also examined as: very mild, mild, moderate, severe. All the data obtained was tabulated, and then subjected to statistical analysis using the Statistical Software Package (SPSS).

OBSERVATIONS AND RESULTS

In the present study, out of 150(100%) primary OSCC cases, maximum cases i.e. 121(80.67%) were in the age range 40-60 years. 16(10.67%) cases were between the age 20-40 years and 13(8.67%) cases were between the age 60-80 years. Out of 50(100%) cases of secondary OSCC cases, maximum cases 35(70%) were in the age range 40-60 years. 8(16%) cases were between the age 20-40 years and 7(14%) cases were between the age 60-80 years. (Table I) In the present study, out of total 150(100%) primary OSCC cases, 108(72%) cases were males and 42(28%) cases were females. Out of 50(100%) secondary OSCC cases, 39(78%) cases were males and 11(22%) cases were females. (Table II)Out of 150(100%) primary OSCC cases, maximum i.e. 58(38.67%) cases were seen on buccal mucosa, followed by lateral border of tongue showing 45(30%) cases and least common site being retro molar trigone region showing 4(2.66%) cases. Similarly, out of 50(100%) secondary OSCC cases, maximum i.e. 26(52%) cases were seen on buccal mucosa, followed by alveolar region showing 9(18%) cases and least common site being floor of the tongue showing 1(2%) case. (Table III)Tumor differentiation pattern assessed using Broder's system of grading showed that 60(40%) OSCC cases of primary tumors were well differentiated, 28(18.67%) OSCC cases were moderately differentiated and 62(41.33%) OSCC cases were poorly differentiated. About 27(54%) OSCC cases were well differentiated, 6(12%) OSCC cases were moderately differentiated and 17(34%) OSCC cases were poorly differentiated in secondary tumor category. Thus, we found that, of the total 200(100%) OSCC cases including both primary and secondary tumor groups, 87(43.5%) cases were well differentiated OSCC, 34(17%) cases were moderately differentiated OSCC and 79(39.5%) cases were poorly differentiated OSCC cases. (Table IV)Connective tissue status for primary tumors showed the predominance of loose type 39(62.90%), 42(70%), 15(53.57%) in well, moderate and poorly differentiated OSCC and least was variable type 3(4.84%), 3(5%) in well and moderately differentiated OSCC and hyalinised type 2(7.15%) in poorly differentiated OSCC cases. The same was true with the secondary tumor with loose type predominating 18(66.67%), 12(70.59%) in well and poorly differentiated OSCC and variable type 3(50%) in moderately differentiated OSCC. Least predominant was variable type 4(14.81%) in well differentiated OSCC, hyalinised type1 (16.67%), 2 (11.76%) in moderately differentiated and poorly differentiated OSCC. On application of Fisher's exact test (P = 0.01*), highly statistical significant difference was observed between the two groups. Thus, loose type of connective tissue was most prevalent type whereas variable type of connective tissue was least prevalent in both primary and secondary OSCC cases. (Table V)(Graph V)(Table V) (Graph V)Status of inflammation in the primary tumor group showed a predominance of moderate grade of inflammation 31(63.27%), 33(55.93%) in well and moderately differentiated OSCC while severe type was predominant in poorly differentiated OSCC. Severe grade inflammation 4(8.16%), 3(5.08%) was the least type in well and moderately differentiated OSCC and mild was least in poorly differentiated OSCC. In secondary tumor group, moderate grade of inflammation predominated 8(47.06%), 16(59.26%) in well and moderately differentiated OSCC and severe predominated 5(83.33%) in poorly differentiated OSCC cases. Very mild grade 2(11.76%), 1(16.67%) was least in well and poorly differentiated OSCC and mild grade 4(14.81%) 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*). Thus, most of the inflammatory response was moderate type and very mild type was least seen in both primary and secondary OSCC. (Table VII)(Graph VII)(Table VIII) (Graph VIII)

Table I: Age-wise distribution of Primary and Secondary OSCC cases
OSCC- Oral Squamous cell carcinoma

Age	Primary OSCC cases	Secondary OSCC cases	Total
20 – 40 yrs	16(10.67%)	8(16%)	**24(12%)**
40 – 60 yrs	121(80.67%)	35(70%)	**156(78%)**
60 – 80 yrs	13(8.67%)	7(14%)	**20(10%)**
Total	**150 (100%)**	**50(100%)**	**200(100%)**

Table II: Gender-wise distribution of Primary and Secondary OSCC cases

Gender	Primary OSCC cases	Secondary OSCC cases	Total
Male	108(72%)	39(78%)	**147(73.5%)**

Female	42(28%)	11(22%)	**53(26.5%)**
Total	**150(100%)**	**50(100%)**	**200(100%)**

OSCC- Oral Squamous cell carcinoma

Table III: Site of occurrence of Primary and Secondary OSCC cases

Site of occurrence	Primary OSCC cases	Secondary OSCC cases	Total
Buccal Mucosa	58(38.67%)	26(52%)	**84(42%)**
Lateral border of tongue	45(30%)	7(14%)	**52(26%)**
Floor of mouth	6(4%)	1(2%)	**7(3.5%)**
Alveolus	24(16%)	9(18%)	**33(16.5%)**
Lip	6(4%)	3(6%)	**9(4.5%)**
Retromolar trigone	4(2.66%)	3(6%)	**7(3.5%)**
Others	7(4.67%)	1(2%)	**8(4%)**
Total	**150(100%)**	**50(100%)**	**200(100%)**

OSCC- Oral Squamous cell carcinoma

Table IV: Distribution of Primary and Secondary OSCC cases according to Broader's grading system

Broader's grading system	Primary OSCC cases	Secondary OSCC cases	Total
Well differentiated	60(40%)	27(54%)	**87(43.5%)**
Moderately differentiated	28(18.67%)	6(12%)	**34(17%)**
Poorly differentiated	62(41.33%)	17(34%)	**79(39.5%)**
Total	**150(100%)**	**50(100%)**	**200(100%)**

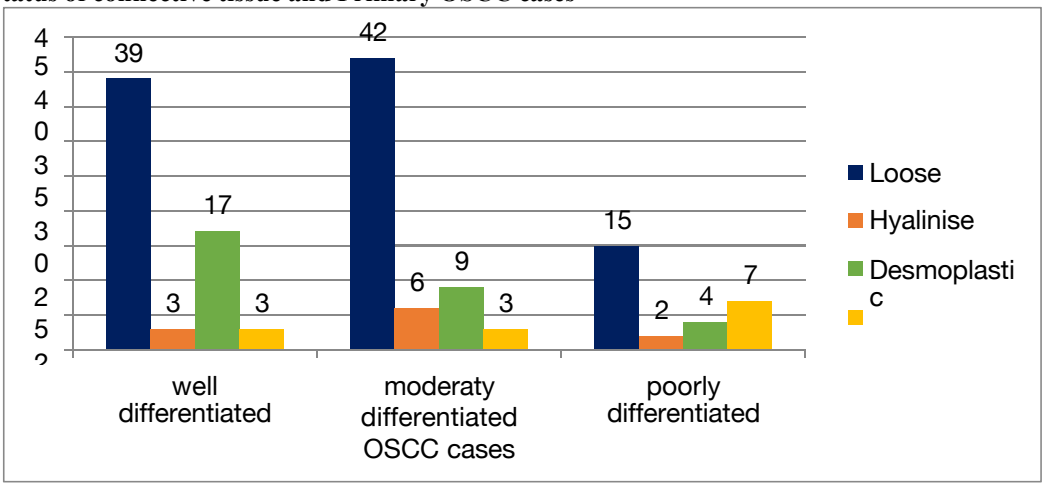
OSCC- Oral Squamous cell carcinoma

Table 5: Comparison of primary tumor with respect to status of connective tissue

Status of connective tissue			
	Well differentiated OSCC cases	**Moderatly differentiated OSCC cases**	**Poorly differentiated OSCC cases**
Loose	39(62.90%)	42(70%)	15(53.57%)
Hyalinised	3(4.84%)	6(10%)	2(7.15%)
Desmoplastic	17(27.42%)	9(15%)	4(14.28%)
Variable	3(4.84%)	3(5%)	7(25%)
Chi Square test	Chi Square value 1.54 (p value 0.01< 0.05, So significant)		
Fisher's exact test	Fisher Value 1.25 (p value 0.03 < 0.05, So significant)		

OSCC- Oral Squamous cell carcinoma

Graph V: Status of connective tissue and Primary OSCC cases



OSCC- Oral Squamous cell carcinoma

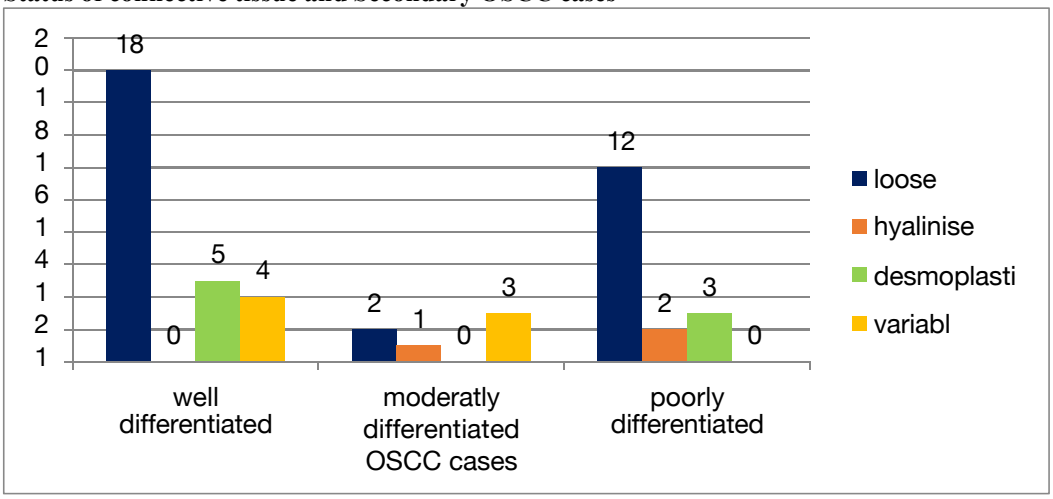
Table VI: Comparison of secondary tumor with respect to status of connective tissue

Status of connective tissue	Well differentiated OSCC cases	Moderately differentiated OSCC cases	Poorly differentiated OSCC cases
Loose	18(66.67%)	2(33.33%)	12(70.59%)

Hyalinised	0(0%)	1(16.67%)	2(11.76%)
Desmoplastic	5(18.52%)	0(0%)	3(17.65%)
Variable	4(14.81%)	3(50%)	0(0%)
Chi Square test	Chi Square value 1.37 (p value 0.03< 0.05, So significant)		
Fisher's exact test	Fisher Value 1.45 (p value 0.02< 0.05, So significant)		

OSCC- Oral Squamous cell carcinoma

Graph VI: Status of connective tissue and Secondary OSCC cases



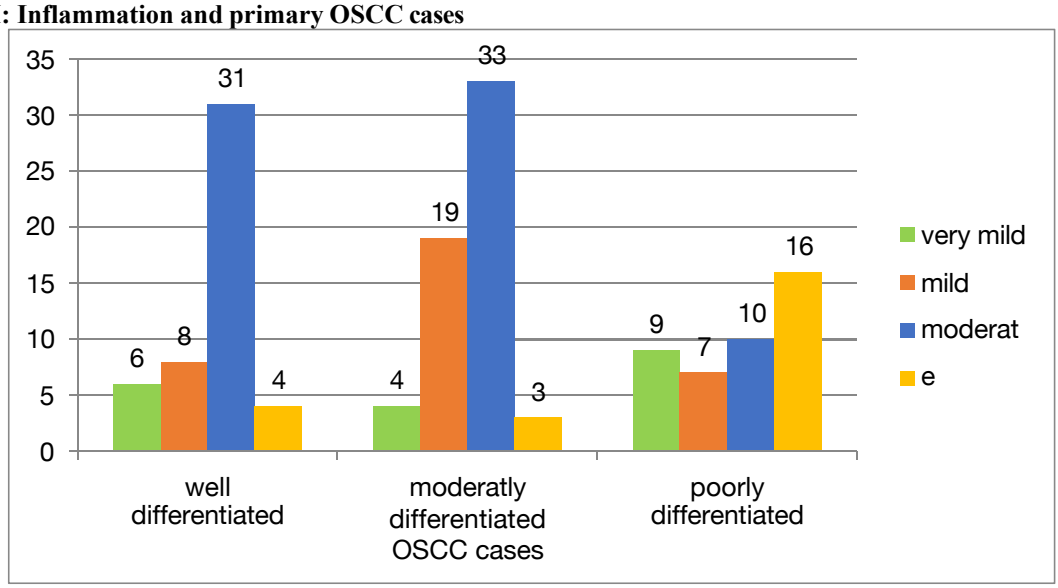
OSCC- Oral Squamous cell carcinoma

Table VII: Comparison of primary tumor with respect to status of inflammation

Status of inflammation			
	Well differentiated OSCC cases	Moderately differentiated OSCC cases	Poorly differentiated OSCC cases
Very mild	6(12.24%)	4(6.78%)	9(21.43%)
Mild	8(16.33%)	19(32.20%)	7(16.67%)
Moderate	31(63.27%)	33(55.93%)	10(23.81%)
Severe	4(8.16%)	3(5.08%)	16(38.09%)
Chi Square test	Chi Square value 2.81 (p value0.000< 0.05, So significant)		
Fisher's exact test	Fisher Value 2.91 (p value 0.000 < 0.05, So significant)		

OSCC- Oral Squamous cell carcinoma

Graph VII: Inflammation and primary OSCC cases



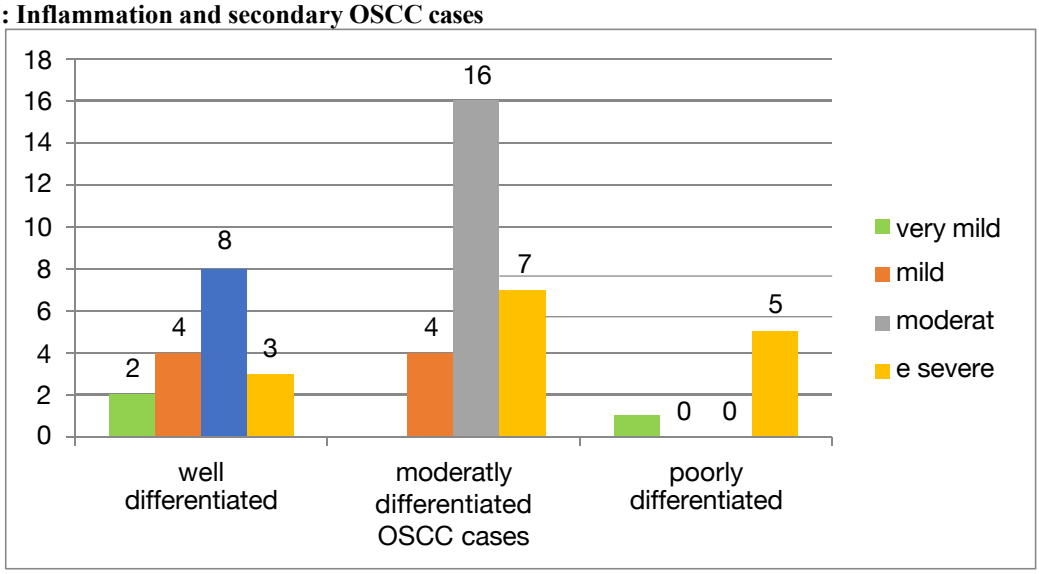
OSCC- Oral Squamous cell carcinoma

Table VIII: Comparison of secondary tumor with respect to status of inflammation

Status of inflammation	Well differentiated OSCC cases	Moderately differentiated OSCC cases	Poorly differentiated OSCC cases
Very mild	2(11.76%)	0(0%)	1(16.67%)
Mild	4(23.53%)	4(14.81%)	0(0%)
Moderate	8(47.06%)	16(59.26%)	0(0%)
Severe	3(17.65%)	7(25.93%)	5(83.33%)
Chi Square test	Chi Square value 1.58 (p value 0.01< 0.05, So significant)		
Fisher's exact test	Fisher Value 1.92 (p value 0.000 < 0.05, So significant)		

OSCC- Oral Squamous cell carcinoma

Graph VIII: Inflammation and secondary OSCC cases



OSCC- Oral Squamous cell carcinoma

DISCUSSION

Margin status is the main prognostic factor guiding adjuvant treatment (postoperative radiotherapy and chemotherapy) decisions in early stage and node negative patients. However, some patients do not show good survival despite of proper treatment received. To evaluate this poor tumor biology, various prognostic parameters have been proposed. Tumor grade, perineural invasion (PNI), lymph vascular invasion (LVI) are well studied, whereas some novel ones are the worst pattern of invasion (WPOI), lymphocytic host response (LHR), status of connective tissue, smallest cell nest size (sCNS), and tumor budding (TB).(2) Invasion is one of the important hallmarks of cancer. Thus, our study focused on ITF with different novel parameters POI, Status of connective tissue and Inflammatory host response in both primary and secondary OSCC cases.In our study we found that most common age range of primary and secondary OSCC cases was 40 to 60 years age. Similar results were found by Juan Carlos cuevas-Gonzalez(1), T. Jeelani(3) and YOPOVINU Rhutso(4) in their studies where subjects show maximum occurrence in fifth and sixth decade of life. In our study, males had higher occurrence of OSCC as compared to females in both primary and secondary OSCC cases. This can be attributed to habit association which is more prevalent among males than females in south Asian countries.Most common site of occurrence was buccal mucosa in both the genders. Also both primary and secondary tumors showed buccal mucosa most common site. It is followed by lateral border of the tongue, whereas least common site was floor of the mouth and retro molar trigone. Similar results were found by B.S.M.S. Siriwardena(5) and acharya et al(6) in their studies. Whereas Deval parikh(2) and T. Jelani(3) found that most common site of occurrence was tongue in their study. Site predilection can also be attributed to risk habits. The frequent site of occurrence is buccal mucosa in south Asian countries where betal quid chewing is more common. And in western countries, smoking and alcohol are more common habits and thus floor of mouth and tongue are more commonly affected. Other than habit association, many other multiple etiological factors can be considered to be responsible for this difference.Broader's grading system being simple to use and easy, we have evaluated OSCC tumors according to this system. In our study we found that in primary OSCC cases, maximum cases were poorly differentiated OSCC cases whereas in secondary OSCC cases, maximum cases were well differentiated OSCC cases. Nadaf(7) in their study found maximum cases were well differentiated both in primary tumor and secondary tumor group. This difference can be because of

difference in sample size used by different authors in their different studies.Mostly malignant human neoplasm has heterogeneous cell populations that usually show different biological behaviours. It is stated that poorly differentiated cells within the superficial parts of the tumour do not necessarily reflect aggressiveness of tumour, and grading of these parts may thus not be an indicator for the clinical behaviour of the tumour.Cancer is a condition that emerges from interaction between tumor–host microenvironment where malignant tumor cells recruit vasculature and stroma through the production and secretion of growth factors and chemokines. The locally activated host microenvironment controls the proliferation and behavior of the tumor cells. It also creates a permissive field to supply nutrients by angiogenesis and provides a way for metastasis through the vascular system.(3)In our study, we found that loose type of connective tissue was predominantly seen in all three grades of primary as well as secondary OSCC cases at ITF. Similar results were found by Nadaf(7) in their study where loose type of connective tissue was predominantly seen. This can be one of the important parameter for morphologic diagnosis of an invasive tumor and may be the result of a complex cross-talk between the tumor cells and the host connective 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 in a study by Spiro RH(8) and the uterine cervix indicating that poorly differentiated tumors may induce a strong remodeling process in the juxtratumoral stroma in a study by Horn LC(9). This can be attributed to increased MMP production in the connective tissue which leads to collagen breakdown because of which loose type of connective tissue is seen. 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.(6)Moderate inflammation was seen to be predominant in all grades of both primary and secondary OSCC cases at ITF in our study. Similar results were found by Nadaf(7) in their study where moderate inflammation was predominantly seen. Also T. Jeelani found moderate growth of inflammation in their retrospective study of 60 cases of primary OSCC.(3) Deval parikh(2) in their study concluded that in addition to tumor morphological parameters, lack of host-immune lymphocytic response is a significant marker of poor tumor behavior. Thus inflammation has significant role in controlling tumor behavior.It has also been suggested that lymphocytic response can promote tumourigenesis by production of several growth promoting signalling molecules (EC GF, VEGF, FGF2, 2, chemokines and cytokines). Lymphocytic response can also potentially eradicate tumor cells. Hence the immune and inflammatory response may have both tumor promoting and anti-tumor effects, acting as a double-edged sword. In a study by Chatzistamou et al.(10) on oral tongue SCC, high-density inflammatory host response was found to have better survival rate. Lundqvist et al.(11) found a correlation between density of host response and favorable response to radiotherapy in tongue SCC. B.S.M.S. siriwedena(5) in their study found statistically significant association between the 3-year survival rate and lymphocytic host response. Therefore, it can be assumed that the antitumor characteristics of inflammatory cells might be a reason for better prognosis of patients according to some researchers.Therefore, grading status of inflammation depending on its density without knowledge of the composition of the infiltrate may be potentially misleading. The precise composition of the lymphocytic host response is not investigated here or in other studies.(9) We propose further research to be conducted in this regard investigating the composition of inflammatory infiltrate in underlying connective tissue.Thus, Cancer cells at the invasive tumor front (tumor-host interface) have been an area of interest for histopathological research nowadays. The most aggressive cells of the tumor reside at ITF and show prognostic significance with relation to PPOI and mainly WPOI, lymphocytic host response and status of underlying connective tissue. Thus we propose to add these parameters in histopathological reporting by the pathologist which will help the surgeon to formulate proper treatment planning with giving less chance for recurrence of the disease.

CONCLUSION

Histopathological parameters of tumor invasive front are evaluated by the pathologist at the time of diagnosis of the case. But it is yet not a part of the microscopic description that the treating physician receives. Thus we propose evaluation and reporting of ITF in the pathology report so that it can guide the treating oncologist to establish the therapeutic route for each patient. Inflammation acting as a double-edged sword, it is necessary to evaluate the composition of inflammation to know whether it is acting as pro-tumorigenic or anti-tumorigenic factor. So we propose that further studies should be carried out to know the role of inflammation and its released chemokines in progression of OSCC cases.

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