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Prognostic impact of tumor-stroma ratio in oral squamous cell carcinoma - A pilot study

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A B S T R A C T

Background: Several prognostic indicators have been used for many decades in an attempt to predict clinical behaviour of Oral Squamous Cell Carcinoma (OSCC). The prognostic value of TSR is yet to be explored in OSCC. Hence, the aim of the present study was to evaluate the prognostic value of TSR in OSCC patients.
Methodology: A cohort of 60 histologically diagnosed cases of OSCC who underwent Radical Neck Dissection was included in the study. TSR was assessed and patients with > 50% intratumor stroma were quantified as the stroma-poor group and those with < 50% as the stroma-rich group.
Results: The parametric tests were performed for the statistical evaluation of TSR with the clinico-pathological variables and the survival. The 3-year overall survival (OS) and disease-free survival (DFS) rates were 95.23% and 69.04%, respectively, in stroma-poor group and 77% and 44%, respectively in the stroma-rich group.
Conclusion: TSR may serve as a reliable histologic prognostic indicator in OSCC and could be used in routine diagnostic pathology.

1. Introduction

Tumor tissue is composed of epithelial cells and stromal cells recruited from normal tissue. In normal tissue, the stroma may act as a barrier in tumorigenesis by constraining tumor cell proliferation. However in tumor tissue, stromal components could facilitate the process of tumor progression. Although the mechanism underlying is still not clear, tumor-associated stroma and cancer-associated fibroblasts have been found to play an important role in tumor progression phases [1]. Recently, as a consequence of the growing interest in the microenvironment, several studies have been conducted to evaluate the ratio of tumor to stroma (TSR) as a reflection of the microenvironment of cancer and survival outcome in Esophageal Cancer, Breast Cancer, Colon Cancer, and Cervical Cancer [2-5]. TSR has been proven to be an independent prognostic factor for these cancers. Tumor–stroma ratio could be easily implemented in routine haematoxylin-eosin stained slides without any special staining methods, and is simple to determine, reproducible, and performed in quick time. Although to our knowledge, the prognostic value of TSR is yet to be explored in OSCC. Hence, the aim of the present study is to assess the prognostic efficiency of TSR in determining the overall survival and disease free survival of the patients with OSCC.

2. Materials and methods

A retrospective study was planned and an approval from the ethical committee was taken (IRB No- 2015/P/OP/42). A total of 60 surgically resected cases of OSCC treated with Radical Neck Dissection (RND) from the year 2013–2016 were selected from the archives of the Department of Oral Pathology and Microbiology, Shri Dharmasthala Manjunatheshwara College of Dental Sciences and Hospital, Dharwad. The clinical details, 3-year disease-free survival (DFS) and overall survival (OS) were collected from the case file of each patient.

Inclusion criteria:

1) Radical neck dissected cases of OSCC
2) Presence of most Invasive tumor front area

Exclusion criteria:

1) Patients received preoperative chemo- or radiation therapy.
2) Presence of known distant metastasis at surgery.
3) Patients with other malignancies in the past and death or recurrence (distant or loco-regional) within 1 month.
4) The lesional tissue not including the most invasive deep front area.

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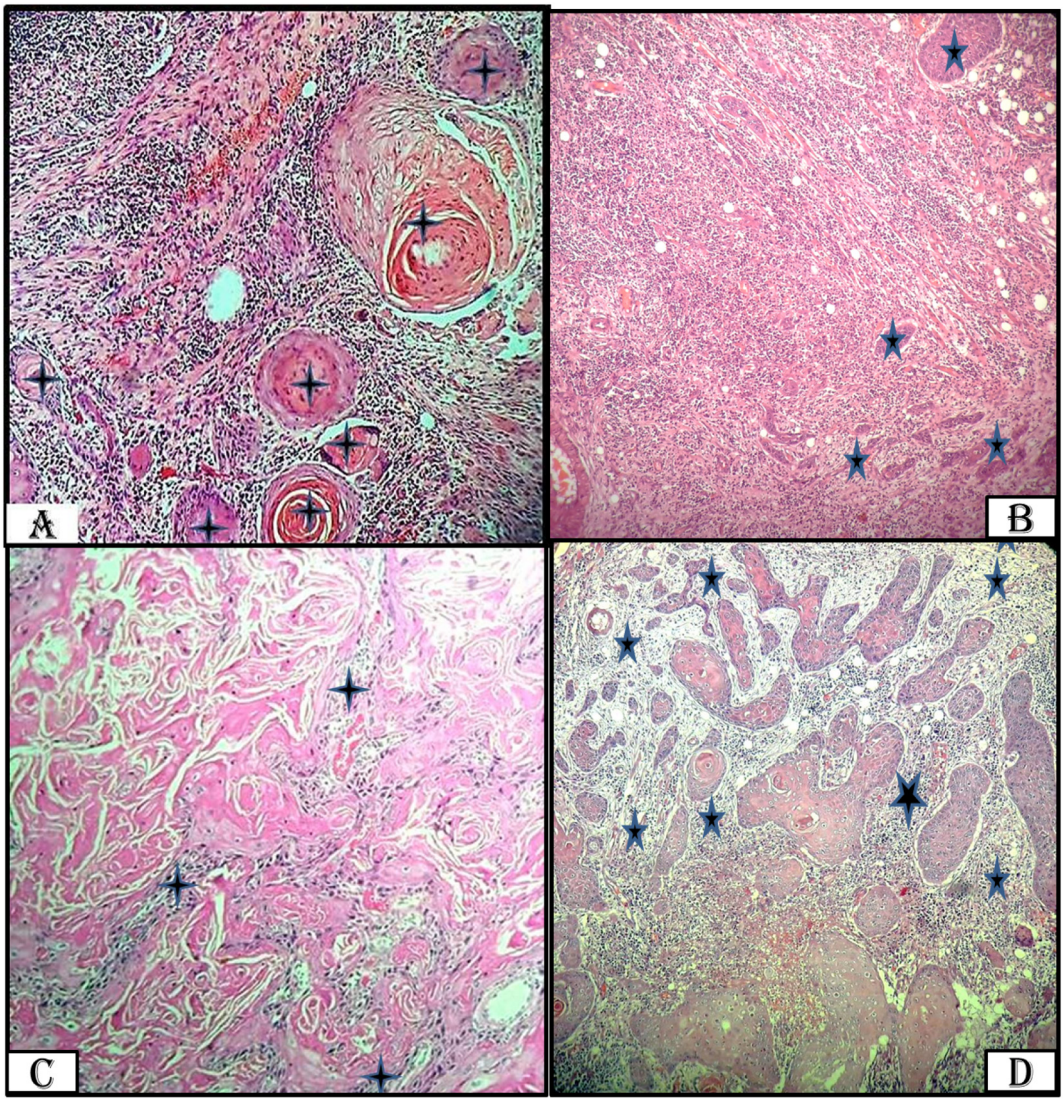

Fig. 1. Haematoxylin and Eosin stained sections of Oral Squamous Cell Carcinoma (OSCC) (original magnification ×10).
A and B: example of stroma-rich (stroma > 50%) (*-tumor)
C and D: example of stroma-poor (stroma < 50%) (*-stroma).

2.1. Histopathological evaluation

Histopathological examination involved routine microscopic analysis of Haematoxylin and Eosin stained sections from the most invasive part of the tumor. Using a 4× objective, the most invasive tumor front of the whole tissue slide was selected. Subsequently using a 10× objective, only those fields were scored where both the stroma and tumor are present and most importantly tumor cells are seen on all sides of the microscopic image field. TSR was visually estimated in a blinded manner by two investigators (KCN and NAS) and scored per tenfold percentage. In case of heterogeneity, the highest stromal percentage was considered decisive.

2.2. Statistical analysis

The cut-off value for the TSR was taken as 50% as previously determined in Colon Cancer [4] and Breast Cancer with the maximum discriminative power [6]. TSR was defined as stroma poor (the proportion of stroma < 50%) or stroma rich (the proportion of stroma > 50%) (Fig. 1). Differences in the clinico-pathological characteristics were assessed using the Chi-square test. The inter-observer variability was analysed using Cohen's Kappa co-efficient. The Cox proportion hazards model was used to determine the hazard ratio of variables on 3 year DFS and OS in univariate and multivariate analysis. Analysis of survival curves was performed using Kaplan-Meier Survival Analysis and survival distributions were evaluated with Log rank statistics. The TSR was then correlated with clinico-pathologic parameters and the disease-free survival (DFS) and overall survival (OS).

3. Results

3.1. Clinicopathological features

60 patients (53 men and 7 women) were included in the present study. The median age of patients was 50 years at the date of surgery. The median follow up time was 36 months (range, 18–48 months). Clinicopathological characteristics of the patients are shown in Table 1.

3.2. Tumor stroma ratio in OSCC

With 4× and 10× objectives, routine Haematoxylin and Eosin stained sections from the primary tumor were analysed for the presence of stromal involvement. TSR was assessed on one section obtained from the most invasive front of the tumor (Fig. 1). TSR was assessed by 2

Table 1
Clinicopathologic parameters in stroma poor and stroma rich group in OSCC.

Parameter		Total no. (%), n = 60	High TSR no. (%), n = 42	Low TSR no. (%), n = 18	p value
Age	< 50 yrs	26 (43.33)	19 (45.23)	7 (38.88)	0.996 (NS)
	> 50 yrs	34 (56.66)	23 (54.76)	11 (61.11)	
Gender	Male	53 (88.33)	36 (85.71)	17 (94.44)	**0.01 (S)**
	Female	7 (11.66)	6 (14.28)	1 (5.55)	
Histologic grade	Well	40 (66.6)	29 (69.04)	11(61.11)	0.84 (NS)
	Mod	20 (33.33)	13 (30.95)	7 (38.88)	
Lymph-node status	Positive	24 (40)	18 (42.85)	6 (33.33)	0.063 (NS)
	Negative	36 (60)	24 (57.14)	12 (66.66)	
Type of growth	Endophytic	45 (75)	30 (71.42)	15 (83.33)	0.99 (NS)
	Exophytic	15 (25)	12 (28.57)	3 (16.66)	
Staging	Stage 1	3 (5)	2 (4.8)	1 (5.6)	0.346 (NS)
	Stage 2	16 (26.66)	13 (38)	3 (16.7)	
	Stage 3	41 (68.33)	27 (64.3)	14 (77.8)	
Inflammatory response	Mild	32 (53.33)	21 (50)	11 (61.11)	0.95 (NS)
	Mod	17 (28.33)	12 (28.57)	5 (27.77)	
	Severe	11 (18.33)	9 (21.42	9 (21.42)	
ECS	Positive	14 (23.33)	9 (21.42)	5 (27.77)	**0.02 (S)**
	Negative	46 (76.66)	33 (78.57)	13 (72.22)	
Habits	Tobacco	51 (85)	35 (83.33)	16 (88.8)	0.958 (NS)
	Combi	9 (15)	7 (16.66)	2 (11.11)	
DOI	1–5 mm	10 (16.66)	7 (16.66)	3 (16.66)	**0.00 (S)**
	5–10 mm	30 (50)	26 (61.9)	4 (22.22)	
	> 10 mm	20 (33.33)	9 (21.42)	11 (61.11)	
POI	Type 1	7 (11.66)	6 (14.28)	1 (5.55)	**0.007 (S)**
	Type 2	18 (30)	15 (35.71)	3 (16.66)	
	Type 3	27 (45)	19 (45.23)	8 (44.44)	
	Type 4	8 (13.38)	2 (4.76)	6 (33.33)	
PNI	Positive	9 (15)	7 (16.66)	2 (11.11)	**0.00 (S)**
	Negative	51(85)	35(83.33)	16 (88.88)	
PVI	Positive	5 (5)	5 (11.90)	0 (0)	**0.00 (S)**
	Negative	55 (95)	37 (88.09)	18 (100)	

Bold data signifies $p < 0.05$.

independent observers, 42 tumors were stroma poor and 18 were stroma rich. Cohen's kappa coefficient revealed an almost perfect agreement between the 2 observers ($\kappa = 0.932$).

3.3. Correlation of TSR with other prognostic factors

Table 1 shows the patient, tumor, and pathological characteristics for the stroma-rich and stroma-poor groups. Significant differences were observed between the stroma rich and stroma poor tumors with respect to ECS, DOI (> 10 mm), POI (Type III), PNI and PVI (Figs. 2, 3). In Cox Multivariate analysis, POI, tumor grading and DOI were observed to be independent prognosticators for OS and DFS. The 3-year overall survival (OS) and disease-free survival (DFS) rates were 95.23% and 69.04%, respectively, in stroma-poor tumors and 77% and 44%, respectively in the stroma-rich tumors. Median OS and DFS of patients in the stroma poor tumors were 40 months (95% CI, 38–41 months) compared with 38 months (95% CI, 36–39 months) in stroma rich tumors. Survival curves for stroma poor and stroma rich tumors are shown in Figs. 4 & 5. The difference of survival curves between the 2 groups was statistically significant with respect to 3 year DFS (Table 2).

4. Discussion

In the past few decades tumor surrounding stroma has received

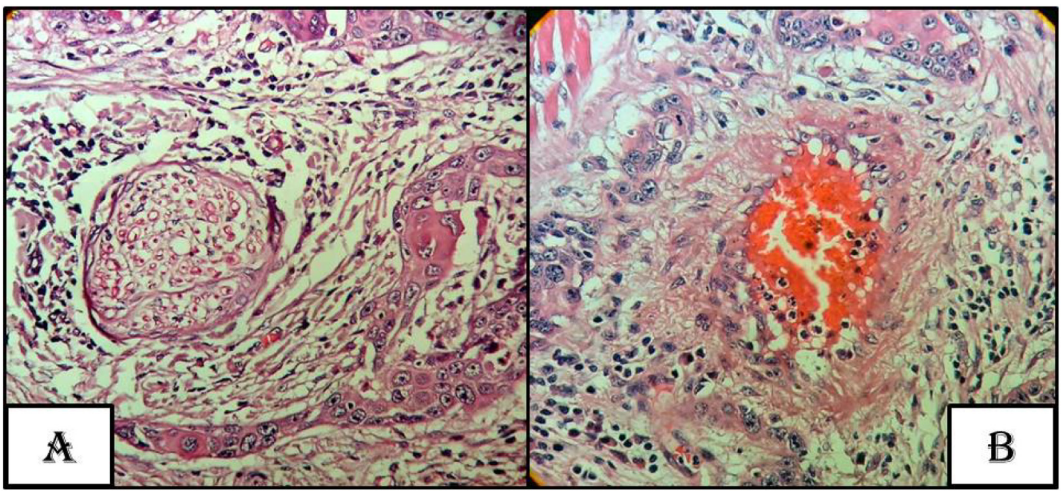

Fig. 2. Haematoxylin and Eosin stained sections showing Perineural invasion (A) and Perivascular invasion (B) in Oral Squamous Cell Carcinoma (OSCC) (original magnification ×40).

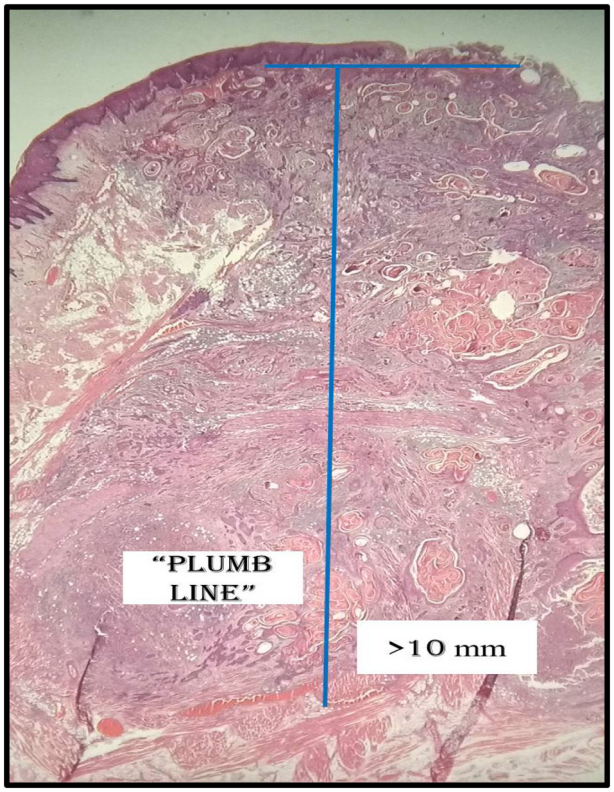

Fig. 3. Depth of Invasion measured by drawing the "Horizon" that is at the Level of the Basement Membrane Relative to the Closest Intact Squamous Mucosa. The greatest invasion is measured by dropping a "plumb line" from the horizon.

increased consideration with some evidence that cancer initiation, growth and progression are dependent on tumor microenvironment [7] and tumor stroma is an important part of it. This can be illustrated by the "Seed and Soil" concept, where the cancer cells called "Seeds", survive in a highly complex microenvironment of the surrounding stroma called – "Soil". The stroma surrounding the cancer cells is not passive and plays an active role in supporting and nourishing tumor parenchyma. Lately, tumor-stroma ratio (TSR) has been utilized by researchers in various cancers affecting the human body, and has found it to be efficient in determining the prognosis of the patients. TSR as defined by Wilma Mesker is the amount of stromal component that surrounds the cancer cells.

In the literature search conducted, this was the first attempt of assessing the role of TSR in OSCC. In the present study, we analysed the TSR in 60 cases of OSCC. The optimal threshold level of TSR was determined on the basis of a maximum discriminating power for DFS and OS, according to Mesker et al. [6]. Therefore, all patients were classified as "stroma-rich" or "stroma-poor" according to the proportion of stroma $\geq 50\%$ or $< 50\%$, respectively.

Histopathologic evaluation of OSCC in terms of prognosis in the past have included several parameters, such as histopathologic grading, Depth of Invasion (DOI), Pattern of Invasion (POI), Extracapsular spread (ECS), Perineural invasion (PNI) and Perivascular invasion (PVI) [2,6]. All these factors were included in the present study and we found that there was a significant difference in the stroma rich and stroma poor group of patients with respect to ECS, DOI (> 10 mm), POI (Type III), PNI and PVI. Similar results were noted by Wang et al. [2], where lymph node status, positive lymph node ratio, pTNM stage, DOI and Radicality of resection were significantly related to 3 year OS in univariate analysis.

In the present study, stroma rich group was associated with a reduced 3 year OS and DFS. However the association was not independent in the multivariable analysis and TSR did not significantly affect the OS and DFS. This difference could be explained based on the difference in the histological grades of OSCC which results in different impact of standard prognostic variables between each histological grade. Similar findings were observed by Pongsuvareeyakul et al. [3] in early stage cervical adenocarcinoma and Chen et al. [8], in Epithelial Ovarian Cancer. The authors noted that although TSR was not an independent prognostic factor but higher stroma-rich proportions were found in patients with advanced stage, LN metastasis and recurrence; showing an adjuvant association between TSR and other factors.

TSR has been utilized as a prognostic factor by other studies. Mesker et al. [6], analysed 122 patients of stage I to III colon carcinoma and found that patients with TSR $<$ 50% showed significant worse OS and DFS. The author concluded that TSR could serve as an independent

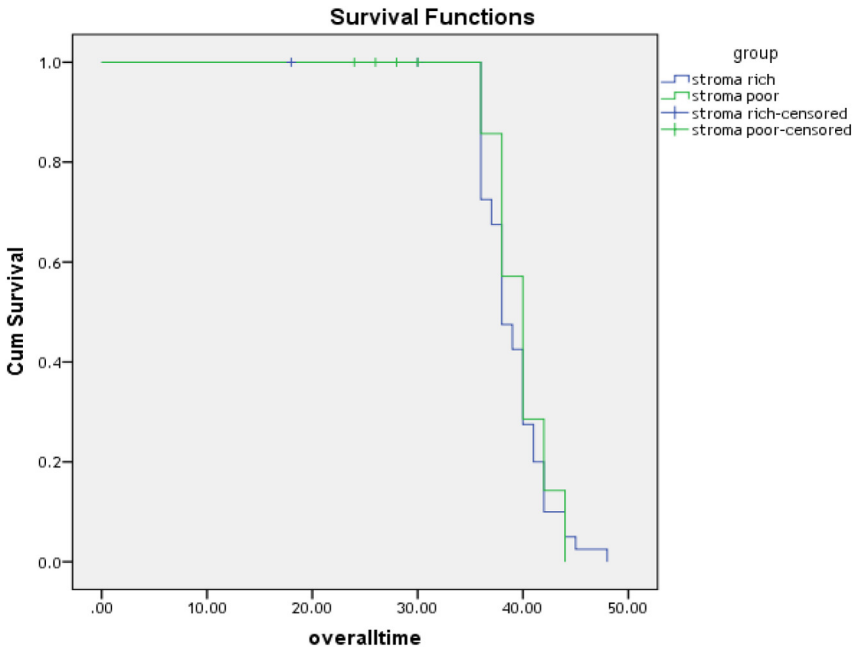

Fig. 4. Survival curve for 3 year overall survival.

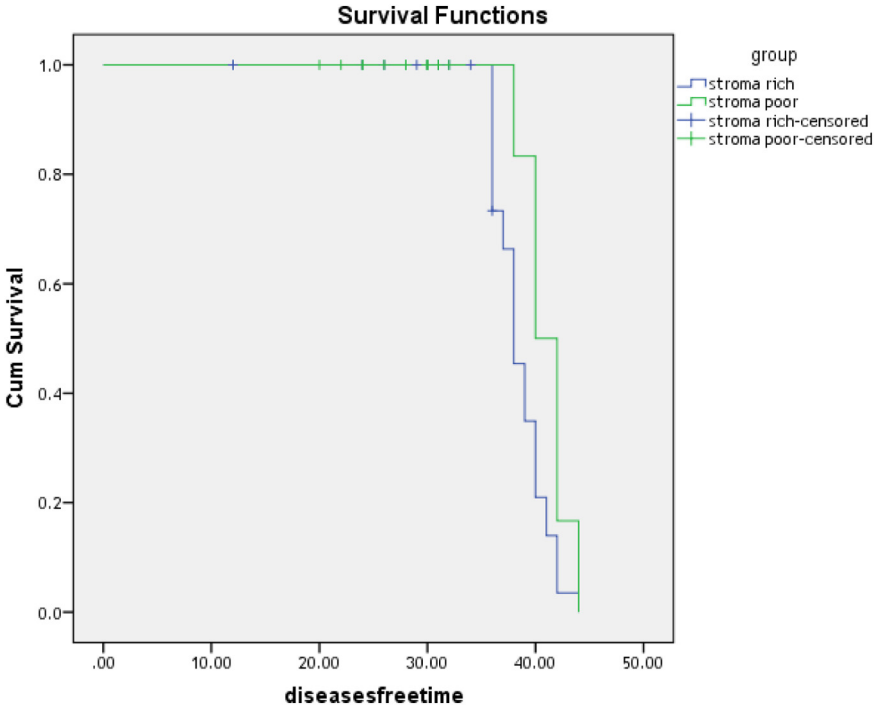

Fig. 5. Survival curve for 3 year disease free survival.

Table 2
3 year overall survival (OS) analysis by Kaplan Meier Curve.

Group	Median				Significance (Log rank test)
	Estimate (months)	Std. error	95% confidence interval		
			Lower bound	Upper bound	
Stroma rich	38.000	0.632	36.762	39.238	**0.565**
Stroma poor	40.000	0.845	38.343	41.657	

Bold data signifies $p < 0.05$.

parameter for prognostication in early-stage colon carcinoma. TSR was also confirmed as an independent and practicable prognosticator in Esophageal Squamous Cell Carcinoma (ESCC), ovarian carcinoma, T1 high-grade bladder cancer and early breast cancer [2,4,8-10].

Compared to the stroma poor group, those of the stroma rich group had a lower 3 year OS (95.23% versus 77%, p = 0.565-Log rank test) and significantly lower 3 year DFS (69.04% versus 44%, p = 0.05-Log rank test) (Table 2 and 3) in the present study. This gives a possibility that TSR could be a prognostic factor in patients with OSCC. The practicable and high reproducibility in evaluating TSR and Kappa value of 0.932 makes TSR an interesting histological prognostic variable in OSCC.

In the current study, other factors like POI, tumor grading, and DOI were seen to be independent prognosticators for a reduced OS and DFS in OSCC. Although TSR was not found to be an independent prognostic factor in the present study, it could be an adjuvant factor for assessing the OS and DFS in OSCC.

Our results have indicated that increased amount of stromal percentage in OSCC can be correlated with a poor prognosis, but not independent of other prognostic factors. Also, TSR can be easily evaluated and performed along with routine pathological examination. As the present study was a pilot study with a relatively smaller sample size, several shortcomings were encountered. Moreover a shorter follow up time of 3 years was assessed.

To conclude, our findings indicate that TSR may play a significant part as a new prognostic factor for OSCC, which is easy to determine on routine HE stained histopathology slides of the resected tumor. Evaluation of TSR can be done pre-operatively in incisional biopsy specimens with a larger sample size in future prospects. In the past, more attention is given to cancer cells for treatment strategies but the dynamic and reciprocal interactions between tumor cells and cells of the tumor microenvironment orchestrate events critical to tumor evolution toward metastasis. Hence, many cellular and molecular elements of the microenvironment are emerging as attractive targets for therapeutic strategies and knowledge about TSR can become influential in these targeted therapies.

Table 3
3 year disease free survival (DFS) analysis by Kaplan Meier Curve.

Group	Median				Significance (Log rank test)
	Estimate (months)	Std. error	95% confidence interval		
			Lower bound	Upper bound	
Stroma rich	38.000	0.588	36.848	39.152	**0.050**
Stroma poor	40.000	1.225	37.600	42.400	

Bold data signifies $p < 0.05$.

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