Pathology - Research and Practice 251 (2023) 154883

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Prognostic evaluation of tumour budding in oral squamous cell carcinoma: Evidenced by CD44 expression as a cancer stem cell marker

Kochli Channappa Niranjan *, Monica Raj, Kaveri Hallikeri

Department of Oral & Maxillofacial Pathology & Oral Microbiology, SDM College of Dental Sciences & Hospital, Shri Dharmasthala Manjunatheshwara University, Dharwad 580 009, Karnataka, India

ARTICLE INFO

Keywords:
Oral squamous cell carcinoma
Tumor budding
Cancer stem cells
CD44
Immunohistochemistry

ABSTRACT

Objectives: Tumor budding is a sign of invasion and early step for metastasis of many cancers including oral squamous cell carcinoma (OSCC). Evidences suggest the presence of cancer stem cells in tumor buds. CD44 has been reported in tumor growth and metastasis as a cancer stem cell marker in OSCC. The study aims to highlight the prognostic significance of tumor budding in association with CD44 expression as a cancer stem cell marker in OSCC.

Methods: A total of 60 radical neck dissection specimens of OSCC with and without lymph node metastasis were included in the study. The sections were evaluated for TB [Tumor Budding] in H&E and CD44 expression immunohistochemically. OSCC cases were then correlated with clinicopathologic and histomorphologic parameters such as age, gender, habit, site, staging, grading, recurrence, depth of invasion, pattern of invasion, and survival outcomes. Comparison of prognosis and CD44 expression were carried out by statistical methods.

Results: A high TB score was significantly correlated with grading ($p = 0.037$), POI [Pattern of invasion] (0.029), overall survival ($p = 0.047$). CD44 over expression showed strong correlations with POI (1HPF:$p = 0.037$;10HPF: $p = 0.027$), grading ($p = 0.037$), and overall survival ($p = 0.047$). Kaplan-Meier analysis revealed overall survival advantage for LTB [Low TB] (85 %) with OSCC compare to HTB [High TB] (75 %) for $>$ 36 months.

Conclusion: Assessment of TB is effective in predicting prognosis of OSCC. Although CD44 expression has demonstrated strong prognostic influence, there were significant differences in its expression with the parameters.

1. Introduction

Oral squamous cell carcinoma (OSCC) accounts for 90 % of malignancies in the head and neck region, making it the sixth most common human malignancy [1]. The overall survival of these patients shows no substantial improvement, with 5-year survival rates remaining at around 50 % [2]. Even, after the standard therapy based on traditional stage-predicting indices based mostly on the TNM criteria and on histological grading [3–5]. Unfortunately, these predictors are subjective and relatively unreliable, as two tumors with identical staging and grading often behave very differently [5]. OSCC is characterised by its variable clinical course [3]. Patients with OSCC exhibit relatively high rates of local recurrence and regional cervical lymph node metastasis (LNM), all of which contribute to significant morbidity and mortality [1]. This has emphasized the need for additional reliable prognostic factors that would enable better categorization of patients based on the biologic aggressiveness of the tumor and eventually guide for more successful and individualized therapy.

Recent studies have identified the presence of tumor budding activity (TBA), defined as small groups of tumor cells, and cell nest size (CNS) as prognostic parameters indicating an unfavorable outcome in OSCC patients [3].

Tumor budding (TB) represents groups of cancer cells that are more invasive than cells in the main tumor mass. It is a specific type of invasive growth in carcinomas characterized by invading single tumor cells or small clusters of tumor cells (<5 cells) at the invasive tumor front (ITF) [7]. TB initially named as 'sprouting' by Imai et al. [8]. Morodomi et al. [9] were the first to report the significance of TB on biopsy samples in relation to LNM.

The oncologic significance of TB along the ITF was first described in colorectal carcinoma and was suggested to correlate with higher malignant potential and biologic aggressiveness of the tumor [10].

* Corresponding author.
E-mail address: niranjankc29@gmail.com (K.C. Niranjan).

https://doi.org/10.1016/j.prp.2023.154883
Received 24 May 2023; Received in revised form 7 October 2023; Accepted 8 October 2023
Available online 17 October 2023

It is a morphologic marker of tumor invasion observed at the invasive margin of tumor nests, and can be defined as isolated or small clusters of undifferentiated cancer cells that detach from neoplasm and migrate into the stroma [10]. It represents two main features of malignancy: loss of cell adhesion and active tumor invasion [12]. Tumor cells that have undergone EMT are histologically characterized as TB. Majority of the studies that assessed TB in OSCC has used one high-power field (HPF) in which the invasive front was scanned and the area with highest number of TB is counted. A method of 1HPF and 10HPF according to Boxberg et al., [6] was used in the present study to score TB. Their scoring criteria had shown significant prognostic value in OSCC.

Recent evidence suggests that cells that undergo EMT gain stem cell-like abilities and can be referred to as cancer stem cells (CSC) [3]. CSC reveals stem cell like characteristics and appears to participate mainly in tumor initiation, progression, metastasis, recurrence and imparting resistance to conventional therapy [13]. These are deemed as the "drivers" of the tumorigenic process [14]. Cluster differentiation 44 (CD44), aldehyde dehydrogenase (ALDH) and CD133 have been successfully used to identify highly tumorigenic CSCs in OSCC [1].

One of the characteristics of CSC is their strong expression of CD44, identifying them as stem cell-like cells [3]. CD44, a transmembrane glycoprotein has various functions in cell division, migration, adhesion and signalling [16]. The principal ligand of CD44 is hyaluronic acid (HA), an integral component of the extracellular matrix. CD44 interaction with HA plays a crucial role in cell invasiveness.

CD44 regulates cell proliferation, survival, and interstitial migration, functions that are pivotal for the growth of tumours and for tissue invasion. In addition, CD44 expression has been linked to EMT [3]. Boxberg et al. has demonstrated strong associations of CD44 overexpression at the ITF with poor histopathological differentiation, high TBA and single cell invasion as signs of EMT. In addition, it was also identified as an independent prognostic factor for poor overall survival (OS), disease-specific survival (DSS), and disease-free survival (DFS) in subset of advanced OSCC [3].

The exact mechanisms by which tumor buds are responsible for aggressive behaviour, and the presence of CSC-related properties in these budding cells is little known till date. Also, the impact of CD44 expression on the prognosis of OSCC remains uncertain, with contradictory findings. Therefore, the aim of the study was to assess the intensity of TB and its prognostic significance in OSCC patients. Additionally, immunohistochemistry (IHC) for CD44 was performed to analyze CSC-like feature in budding cells at the ITF.

2. Materials and methods

A retrospective study was done to determine the expression of CD44 as a Cancer Stem Cell (CSC) marker in histopathologically diagnosed cases of Oral squamous cell carcinoma (OSCC). Approval for the study was obtained from the Institutional Review Board (IRB) committee of this institution (IRB. No.2018/P/OP/59). A study group of 60 OSCC cases, out of which 30 cases with Lymph node metastasis (LNM) and 30 cases without LNM were selected based on the inclusion and exclusion criteria. The clinical, demographical, treatment, survival details and tissue blocks were archived from the patient database of the department and Craniofacial unit (CFU), SDM College of Dental Sciences & Hospital, Dharwad.

2.1. Methodology

Hematoxylin and eosin (H&E) stained sections of the lesion proper from radical neck dissection specimens of 60 OSCC cases were assessed for histopathological features like degree of differentiation, pattern of invasion (POI), depth of invasion (DOI), host response, type of stroma, perineural invasion (PNI), perivascular invasion (PVI) and tumor budding (TB). Representative tissue sections were subjected to immunostaining for CD44. A polymer detection system containing DAB (diamino benzidine) chromogen was used as a visualization kit for positive immunostaining. Standardized immunohistochemistry (IHC) procedure according to the guidelines given by the manufacturer (Biogenex,USA) was followed.

2.2. Histopathologic evaluation

Hematoxylin and eosin (H&E) stained sections of the lesion proper (60 OSCC cases) were taken to assess histopathological features like degree of differentiation, POI, DOI, PNI, PVI, host response, stromal content, and tumor budding activity (TBA).

2.2.1. Evaluation of tumor budding

All sections were examined and scored for the TB and CNS by two observers blinded to clinical data and outcome. The 'branching' of small tumor nests consisting of < 5 tumor cells into the surrounding tissue next to the tumor was defined as 'tumor budding'. Budding was assessed in areas showing maximal TBA and was scored separately in one high-power field (HPF; 40x) displaying the highest TBA and in 10 HPFs. In one HPF, low TBA was defined as 1–4 budding nests and high TBA as ≥ 5 budding nests. In 10 HPFs, low TBA was scored if 1–14 and high TBA if ≥ 15 budding nests were detectable. CNS was assessed at the invasive margin and within the tumor center was scored as follows: clusters composed of > 15 tumor cells classified as large nests, clusters of 5–15 cells as intermediate (small) nests, and single-cell invasion as discohesive tumor cells which shows no evidence of nested architecture.

2.2.2. Evaluation of CD44 expression

CD44 staining is exclusively membranous [Fig. 1]. The assessment of CD44 expression in budding cells was performed using the same method as for the evaluation of budding grade, described above. After scanning of each CD44 positive section under 40x magnifications, the field with the highest number of immunostained budding foci was chosen. Low grade was defined < 5 CD44-positive budding foci and high grade as ≥ 5

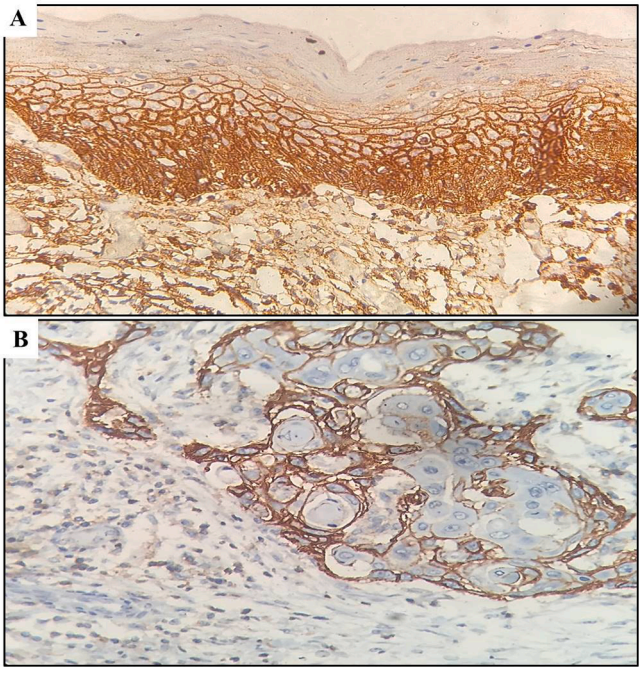

Fig. 1. [A] CD44 immunoexpression pattern in normal adjacent epithelium. (DAB chromogen, CD44 monoclonal antibody, 400x). In normal mucosa, the expression was detected as membranous protein localized on the surface of epithelial cells. [B] CD44 immunohistochemical staining showing membranous expression in the peripheral cells of the tumor islands of OSCC.(DAB chromogen, CD44 monoclonal antibody, 400x).

CD44 positive budding foci [Fig. 2].

2.2.3. Scoring of CD44 staining

CD44 staining is exclusively membranous. Scoring was carried out with a method developed for the immunoreactivity of hormone receptors in breast cancers, separately evaluating the invasive front and tumor core.[66] The percentage of positive tumor cells (PCs) or staining intensity (SI) were classified as follows: score 0, no immunoreactivity; score +1, ≤10 % PCs; weak SI with score 2+, 10–50 % PCs; moderate SI with score 3+, >50–80 % PCs; strong SI with score 4+, >80–100 % PCs. The product of PCs and SI indicated the immunoreactivity score (IRS). An IRS of 9–12 was defined as CD44 high and an IRS of 0–8 as CD44 low.

3. Statistical analysis

A statistical analysis was carried out using SPSS 20.0 (SPSS, Chicago, IL, USA). Correlation of TB with clinicopathologic and histolopathologic parameters were done using $\chi 2$ test. Disease free survival (DFS) and overall survival (OS) according to TB and CD44 expression were determined using the Kaplan-Meier method and log rank test. P-values of < 0.05 was considered to be statistically significant.

4. Discussion

The importance of TB in cancer prognosis has been studied widely particularly in colorectal cancer [33], oesophageal cancer [34], pancreatic cancer [35], breast cancer [36] and lung cancer [37], where it has been reported as a promising prognostic marker. A significant correlation between high tumor budding count and the presence of LNM is one of the most important findings observed in OSCC and many other cancers [38].

4.1. Assessment of TB in OSCC

The definite implementation of TB assessment depends on a selected, internationally accepted scoring system. However, scoring systems of TB are different in reports on several cancers. There is no consensus cut-off point for risk grade for the number of tumor buds even in oral and HNSCC. In spite of this, a cut-off point of 5 buds in 1HPF (40x) (Low: <5buds, high: ≥5buds) is widely accepted by many investigators [19]. The 1 HPF and 10 HPF methods showed excellent inter-observer reproducibility. While the 10 HPF and 1 HPF methods appear to be the only ones revealing the prognostic significance of budding, the authors recommend using the 10 HPF over the 1 HPF method, as it provides superior inter-observer reproducibility due to increased sampling of the region of interest, which increases statistical significance and, therefore, consistency of the findings. However, the best cut off value for stratifying patients under these two methods remains an open question [18].

The frequency of TB varies from 20 % to 89 % widely in various cancers [4]. Wang et al. [12] demonstrated a frequency of 71.7 % in TSCC with HTB at 48.3 %. Almangush et al. [11] reported 34 % HTB in early TSCC. Almost 98.3 % tumors in the current study showed TB with a mean of $11.28 = 10.64$ and $11.58 + 10.62$ using H&E and CD44, respectively (Table 1). Among these, 70 % of the samples were having HTB.

4.1.1. Correlation of TB with clinicopathologic characteristics using H&E in 1HPF and 10HPF

[Table 2] On evaluating TB in relation to the clinicopathological variables, HTB was seen to occur in older age group of > 40 years. This is in contrast to reports submitted by Angadi et al. [4] who found an association of HTB in younger patients. This could be due to more number of subjects who belong to > 40 years in our study group. Studies involving multivariate analysis have confirmed that TB is independent of clinicopathologic parameters. Since number of male subjects was

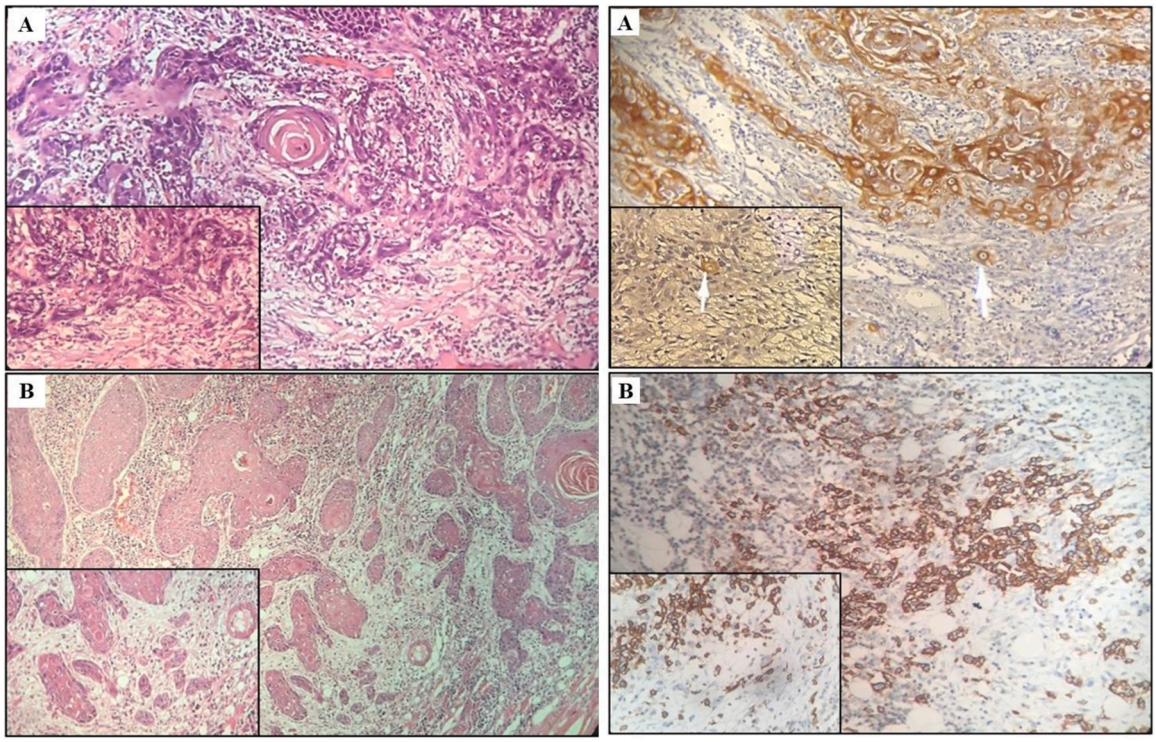

Fig. 2. Left photomicrograph showing tumor budding [A] Low (<5 buds) and [B] High (≥5 buds) at the invasive tumor front of OSCC. (H&E, 200x, 400x inset). Right photomicrograph showing tumor budding at the invasive tumor front of OSCC. [A] Low (<5 buds) and [B] High (>5 buds). (DAB chromogen, CD44 monoclonal antibody, 200x, 400x inset).

Table 1
Correlation of TB assessment in both H&E and CD44 expression of 60 OSCC cases.

	N	Mean ± SD	Mean difference ± SD	t	P VALUE
TB-H&E number	60	11.28 ± 10.64	-0.3 ± 2.53	-0.92	0.361
TB -CD44 number	60	11.58 ± 10.62			

P value < 0.05 [TB-Tumor budding; H&E-Hematoxylin & Eosin; OSCC-Oral squamous cell carcinoma]
The mean values of TB in CD44 (11.58 ± 10.62) was higher in comparison with the mean values of TB in H&E (11.28 ± 10.64) with a difference of 0.3 and is statistically not significant with a p value of 0.361.

more in the study group, TB was observed more among males. HTB activity was observed predominantly associated with habit-oriented patients. This can also be linked to male patients being more inclined to habit-association.

TB was more commonly seen in carcinomas arising from tongue and buccal mucosa (BM) [4]. In the current study, budding was observed predominantly in the order of frequency of site of involvement, i.e, budding was found more in lesions affecting BM (57.1 % in 1HPF; 57.9 % in 10HPF) followed by tongue and gingiva-buccal sulcus / retro-molar (GBS/RM) region (21.4 % in 1HPF and 21.1 % in 10HPF) each. The combination of tumors from different subsites of the oral cavity in the study could be a disadvantage due to which the results lack statistical significance.

Wang et a. [12] studied 230 patients with tongue SCC to evaluate TB and demonstrated a frequency of 71.7 % in tongue SCC with HTB accounting for 48.3 %. Their results showed that TB was linked with tumor size, clinical stage, differentiation and LNM and was concurrent with reduced survival. They also stated difficulty in identifying the tumor buds in a subset of poorly differentiated tongue SCC because of the presence of many lymphocytes, cancer associated fibroblasts and other stromal cells at the ITF. Similar observations suggestive of links between HTB with advanced tumor stage (Stage II-IV), and well differentiated tumors were noted in the present study. We noted that HTB was more frequently observed in patients with stage III (28.6 % in 1HPF; 23.7 % in 10HPF)and stage IV (21.4 % in 1HPF; 23.7 % in 10HPF) than those in stage I-II, suggesting that the tumors with high grade budding are more aggressive and motile with greater potential to dissociate and metastasize. Similar reports of high-grade budding observed more frequently in patients with T3–T4 stage than in those with T1–T2 stage were given by Luo et al.[10].

A correlation of TB and occult LNM was reported in early stage OSCC by Xie et al., 2015 [39]. Such a finding might indicate that TB is an early step en route to metastasis [17]. Our observations also suggested that TB was present in approximately 50 % cases irrespective of the presence/absence of nodal metastasis. And majority of the LNM positive cases (52.4 %; 50 % in 1 and 10HPF) demonstrated HTB, which eventually gives positive associations of HTB with LNM. Tumour budding is found to be a good predictor of clinically node-negative OSCC cases, further adding its utility as an important histopathological parameter. Angadi et al. [4] reported high-intensity tumour budding to be a strong independent prognostic factor for the prediction of LNM. The prognostic efficacy of TB in early-stage tongue SCC has also been observed by Xie

Table 2
Correlation of TB according to Boxberg's criteria (1HPF and 10HPFs, 40x) using H&E with clinicopathological parameters.

Parameter	Categories	N	TB/HPF (H&E,40x)				TB/10 HPF (H&E,40x)			
			Low N (%)	High N (%)	Chi square	P value	Low N (%)	High N (%)	Chi square	P value
Age	<40	15	5(27.8)	10(23.8)	0.106	0.745	7(31.8)	8(21.1)	0.861	0.353
	>40	45	13(72.2)	32(76.2)			15(68.2)	30(78.9)		
Sex	M	44	14(77.8)	30(71.4)	0.26	0.61	15(68.2)	29(76.3)	0.471	0.492
	F	16	4(22.2)	12(28.6)			7 (31.8)	9 (23.7)		
Habits	Absent	13	4(22.2)	9(21.4)	0.005	0.945	5 (22.7)	8 (21.1)	0.023	0.879
	Present	47	14(77.8)	33(78.6)			17(77.3)	30(78.9)		
Site	BM	35	11(61.1)	24(57.1)	0.181	0.914	13(59.1)	22(57.9)	0.079	0.961
	GBS/RM	12	3(16.7)	9(21.4)			4 (18.2)	8 (21.1)		
	Tongue	13	4(22.2)	9(21.4)			5 (22.7)	8 (21.1)		
	Others	0	0(0)	0(0)			0 (0)	0 (0)		
pT	T1	20	6(33.3)	14(33.3)	2.078	0.556	7 (31.8)	13(34.2)	0.907	0.824
	T2	22	5(27.8)	17(40.5)			7 (31.8)	15(39.5)		
	T3	10	3(16.7)	7(16.7)			4 (18.2)	6 (15.8)		
	T4	8	4(22.2)	4(9.5)			4 (18.2)	4 (10.5)		
pN	N0	32	10(55.6)	22(52.4)	0.264	0.876	11 (50)	21(55.3)	1.13	0.568
	N1	23	7(38.9)	16(38.1)			10(45.5)	13(34.2)		
	N2	5	1(5.6)	4(9.5)			1 (4.5)	4 (10.5)		
pM	M0	55	18(100)	37(88.1)	2.338	0.126	21(95.5)	34(89.5)	0.652	0.419
	M1	5	0(0)	5(11.9)			1 (4.5)	4 (10.5)		
Stage	I	13	3(16.7)	10(23.8)	0.53	0.912	4 (18.2)	9 (23.7)	1.194	0.754
	II	17	6(33.3)	11(26.2)			6 (27.3)	11(28.9)		
	III	17	5(27.8)	12(28.6)			8 (36.4)	9 (23.7)		
	IV	13	4(22.2)	9(21.4)			4 (18.2)	9 (23.7)		
LNM	P	30	8(44.4)	22(52.4)	0.317	0.573	11 (50)	19 (50)	0	1
	N	30	10(55.6)	20(47.6)			11 (50)	19 (50)		
Recurrence	Absent	56	17(94.4)	39(92.9)	0.051	0.821	20(90.9)	36(94.7)	0.328	0.567
	Present	4	1(5.6)	3(7.1)			2 (9.1)	2 (5.3)		
OS	<36 m	38	8(44.4)	30(71.4)	3.951	**0.047***	12(54.5)	26(68.4)	1.155	0.282
	>36 m	22	10(55.6)	12(28.6)			10(45.5)	12(31.6)		
DFS	<36 m	42	10(55.6)	32(76.2)	2.555	0.11	14(63.6)	28(73.7)	0.67	0.413
	>36 m	18	8(44.4)	10(23.8)			8 (36.4)	10(26.3)		
Deaths	Alive	47	16(88.9)	31(73.8)	1.688	0.194	19(86.4)	28(73.7)	1.32	0.251
	Dead	13	2(11.1)	11(26.2)			3 (13.6)	10(26.3)		

The correlation of TB(H&E,40x) in 1HPF and 10HPF using Chi-square test were associated with older age, males, site of involvement, habit, tumor stage, LNM, recurrence and survival (OS/DFS) but did not yield any statistical significance except with OS when evaluated in 1HPF (p = 0.047).
* P value < 0.05 [LNM-Lymph node metastasis; OS-Overall survival; DFS-Disease free survival]

et al., 2015 [39].

Additionally, HTB is correlated with lower OS [10], DFS [40], and higher recurrence [18]. However, we observed budding activity in majority of the tumors in both recurrent/non-recurrent groups. The non-recurrent tumors were found to have LTB (94.4 %; 90.9 % in 1 and 10HPF), inferring low budding is associated with lesser chance of recurrence. Even though the study included less number of recurrent cases (n = 4), majority of them exhibited HTB (n = 3) thereby indicating that HTB is associated with high chances of recurrences.

Regarding patient outcomes, consistent evidence has shown that TB is a novel predictor of worse OS time and an independent prognostic factor [41]. In the present study, our data clearly showed that TB was a good predictor of survival in OSCC. HTB had a poorer survival time than those with budding LTB (with statistical significance of p = 0.047 in 1HPF) [Table 2]. Most of the tumors with HTB were also seen associated with lower DFS (76.2 %; 73.7 % in 1 and 10HPF), therefore confirming TB as a strong predictor of survival outcomes in OSCC. In the present study, HTB was found associated with death and LTB with live cases. The mortality rate was found higher in HTB category than in tumors with LTB. However, the difference between LTB and HTB with respect to survival time (OS/DFS) was not statistically significant; this may have been because of the low incidence of deaths. The present study could confirm that HTB, consistent with previous results, was related with more frequency of LNM, stage, site of involvement, local recurrence, and reduced survival (OS/DFS) in OSCC patients.

4.1.2. Correlation of TB with histopathologic characteristics using H&E in 1HPF and 10HPF

[Table 3] Competing grading systems have been proposed, including a multiparameter grading, and specific investigations of the invasive margin [31], such as keratinization, degree of keratinisation (DK), nuclear pleomorphism (NP), host response (HR). There are no reports of association of TB with DK, NP and HR, however, an attempt was made to correlate TBA with the above parameters in the current analyses. In our study, TB was found significantly more common in keratinizing tumors, which was statistically significant (p = 0.037); and also significantly associated with NP (p = 0.042). Based on DK and its association with TB, it was observed that with the decrease in DK, higher was the budding activity (HTB). TB was found least in lesions with increased inflammatory response; i.e., TB was associated with HR, with most of the tumors with moderate inflammatory response showing LTB (45–55 % cases).

TB is also associated with the worse invasive pattern, which has proven to be the best morphological prognostic indicator in oral cancer [32]. Attramadal et al.[40] reported that POI correlated significantly with TB in OSCC. In present study, tumors with POI type I-broad/-pushing invasion were associated with a favourable prognosis (LTB). Our findings of HTB when correlated with POI, HTB was found most in POI-III (45.2 %) > POI-II (42.9 %) > POI-I (7.9 %). Findings were in agreement to that of previous studies which indicates strong correlations of WPOI (spray-like) with HTB [39].

Various studies in colorectal cancer, ampullary cancer, rectal cancer have shown correlation of TB with DOI [4]. The association of TB with DOI has also been confirmed recently by Almangush et al. [11] recently in tongue cancer. This was evident even in the present analyses where HTB significantly correlated with tumors with DOI > 4 mm (85.7 %, 84.2 % in 1HPF and 10HPF). Interestingly, tumour budding (B) was combined with depth of invasion (D) to form the tumour budding and depth of invasion risk model (BD model) for OSCC, which has been associated with a high risk of locoregional recurrence and shortened survival for patients with OSCCs in different studies [42].

The tumor grade (Broder's grading criteria) was expected to be directly associated with TB since both are morphological parameters of

Table 3
Correlation of TB according to Boxberg's criteria (1HPF and 10HPFs, 40x) using H&E with histopathological parameters.

Parameter	Categories	N	TB/HPF (H&E,40x)				TB/10HPF (H&E,40x)			
			Low N (%)	High N (%)	Chi square	P value	Low N (%)	High N (%)	Chi square	P value
Keratin ization	NK	1	0(0)	1(2.4)	6.607	**0.037***	0 (0)	1 (2.6)	4.665	0.097
	K	59	18(100)	41(97.6)			22 (100)	37 (97.4)		
DK	High	19	7(38.9)	12(28.6)	1.91	0.591	9 (40.9)	10 (26.3)	2.726	0.436
	Moderate	21	7(38.9)	14(33.3)			8 (36.4)	13 (34.2)		
	Minimal	18	4(22.2)	14(33.3)			5 (22.7)	13 (34.2)		
	Absent	2	0(0)	2(4.8)			0 (0)	2 (5.3)		
NP	Little	7	4(22.2)	3(7.1)	8.216	**0.042***	4(22.2)	3(7.1)	4.903	0.179
	Moderately-abundant	29	10(55.6)	19(45.2)			10(55.6)	19(45.2)		
	Abundant	23	3(16.7)	20(47.6)			4(22.2)	19(45.2)		
	Extreme	1	1(5.6)	0(0)			0(0)	1(2.4)		
HR	Marked	13	1(5.6)	12(28.6)	4.035	0.133	3 (13.6)	10 (26.3)	2.471	0.291
	Moderate	29	10(55.6)	19(45.2)			10 (45.5)	19 (50.0)		
	Slight	18	7(38.9)	11(26.2)			9 (40.9)	9 (23.7)		
POI	Closed	7	2(11.1)	5(11.9)	7.08	**0.029***	4 (18.2)	3 (7.9)	4.762	0.092
	Finger-like	32	14(77.8)	18(42.9)			14 (63.6)	18 (47.4)		
	Spray-like	21	2(11.1)	19(45.2)			4 (18.2)	17 (44.7)		
DOI	<4 mm	9	3(16.7)	6(14.3)	0.056	0.813	3 (13.6)	6 (15.8)	0.051	0.822
	>4 mm	51	15(83.3)	36(85.7)			19 (86.4)	32 (84.2)		
Grade	Well-diff	48	15(83.3)	33(78.6)	6.607	**0.037***	18(81.8)	30(78.9)	4.665	0.097
	Mod-diff	10	1(5.6)	9((21.4)			2 (9.1)	8 (21.1)		
	Poorly-diff	2	2(11.1)	0(0)			2 (9.1)	0 (0)		
PVI	Absent	49	15(83.3)	34(81)	0.048	0.827	19(86.4)	30(78.9)	0.512	0.474
	Present	11	3(16.7)	8(19)			3(13.6)	8(21.1)		
PNI	Absent	42	14(77.8)	28(66.7)	0.741	0.389	17 (77.3)	25 (65.8)	0.875	0.35
	Present	18	4(22.2)	14(33.3)			5 (22.7)	13 (34.2)		
Stromal content	Very low	10	6(33.3)	4(9.5)	6.314	0.097	7 (31.8)	3 (7.9)	6.045	0.109
	Low	18	6(33.3)	12(28.6)			6 (27.3)	12 (31.6)		
	Moderate	27	5(27.8)	22(52.4)			8 (36.4)	19 (50)		
	High	5	1(5.6)	4(9.5)			1 (4.5)	4 (10.5)		

The correlation of TB/HPF and 10HPF (H&E, 40x) with histopathological parameters using Chi-square test showed significant correlation with keratinization, tumor grade, NP, and with WPOI (using 1HPF). However, in 10HPF, budding had no significant correlation with any of the above histological parameters.

* P value < 0.05 [NK-Non-keratinized; K-Keratinized; DK-Degree of keratinization; NP-Nuclear pleomorphism; HR-Host response; POI-Pattern of invasion; DOI-Depth of invasion; PVI-Perivascular invasion; PNI-Perineural invasion]

aggressiveness in the invasive front. However, this was not observed in the evaluated sample. A possible explanation may be related to the fact that the sample includes a large number of well-differentiated tumors (80 %) as compared to moderately differentiated (16.7 %) and poorly differentiated (3.3 %). Elseragy et al. [43] reported that well--differentiated tumors tend to invade in large islands, but this is not always the case because small clusters of cancer cells can also be present in well-differentiated tumors [44]. We agree with these findings because 78.6 % of our cases had well-differentiated tumors with HTB at the ITF which was statistically significant ($p = 0.037$).

Boxberg et al. [6] found PNI-positive tumors were significantly more frequent in OSCCs with HTB in both IHPF and 10HPF. Reviews by Majumdar et al. [45] have suggested that both PVI and PNI are the known predictors of poor outcome in OSCC patients. In our study, HTB was associated with PVI-positive and PNI-positive tumors in both 1HPF (PVI:19 %; PNI: 33.3 %) and 10HPF (PVI:21.1 %; PNI: 34.2 %), but however did not reach any statistical significance.

Stromal content was found correlated to TBA in the current study, such that the more the stromal content, higher the budding activity, similar to Boxberg et al. [6] where TB correlated significantly with higher stromal content. A recent study by Dourado et al. [46] reported the combination of tumor-stroma ratio and TB resulted in a risk model with a clear discriminatory ability to indicate the prognosis of OSCC patients, especially for cancer-specific survival. The reason for the worse outcome in patients with tumours with a higher proportion of stroma is still unclear, but it is probably related to the interactions between tumour cells and cancer-associated fibroblasts (CAFs).

4.2. Assesment of TB using CD44 in OSCC

In OSCC, CD44 is localised to the membranous expression of the cells in the basal and parabasal layers and around the peripheral cells of the tumor nests [Fig. 1]. Similar findings were observed in the studies by Senbanjo et al. [15], Basakran et al. [16], Andratschke et al. [47]. CD44 was first described as CSC marker in breast cancer and HNSCC, and it has since been used as a CSC marker and prognostic factor for OSCC. CD44 has exhibited positive correlations with tumor recurrence, high-grade SCCs, and poor prognosis [26]. However, its limited usefulness as a CSC marker or prognostic factor in OSCC was also reported [20].

In the present study, CD44 expression was evaluated in terms of CD44-low expression and CD44-over expression based on the immunoreactivity score (IRS) developed by Remmele et al. [30] CD44 expression was present in majority of the cases (98.3 %). The staining intensity ranged varied between weak (21.1 %), intermediate (33.3 %), and strong (45.0 %). The percentage of PCs were in the range of 23–91.4 %; no immunoreactivity (3.3 %), 2 + (55.0 %), 3 + (40.0 %), 4 + (1.7 %) cases. Multiplication of these values resulted in an IRS of 0–8 (CD44-low expression) in 68.3 % cases, and an IRS of 9–12 (CD44- over expression) in 31.7 % cases. The above findings were analogous to those observed by Boxberg et al. [3]. On correlation of CD44 expression with TBA, CD44 expression was associated with the presence of TB with a mean value of (11.58 ± 10.62).

4.2.1. Correlation of TB with clinicopathologic characteristics using CD44 in 1HPF and 10HPF

[Table 4] Furthermore, we also evaluated the relationship between CD44 expression and clinicopathological characteristics of patients. No statistical significant associations were observed between CD44

Table 4
Correlation of TB according to Boxberg's criteria (1HPF and 10HPFs, 40x) using CD44 expression with clinico-pathological parameters.

Parameter	Categories	N	TB/HPF (CD44,40x)				TB/10HPF (CD44,40x)			
			Low N (%)	High N (%)	Chi square	P value	Low N (%)	High N (%)	Chi square	P value
Age	<40	15	6(33.3)	9(21.4)	0.952	0.329	8(36.4)	7(18.4)	2.392	0.122
	>40	45	12(66.7)	33(78.6)			14(63.6)	31(81.6)		
Sex	M	44	14(77.8)	30(71.4)	0.26	0.61	15(68.2)	29(76.3)	0.471	0.492
	F	16	4(22.2)	12(28.6)			7 (31.8)	9 (23.7)		
Habits	Absent	13	4(22.2)	9(21.4)	0.005	0.945	5 (22.7)	8 (21.1)	0.023	0.879
	Present	47	14(77.8)	33(78.6)			17(77.3)	30(78.9)		
Site	BM	35	12(66.7)	23(54.8)	1.326	0.515	14(63.6)	21(55.3)	0.889	0.641
	GBS/RM	12	2(11.1)	10(23.8)			3 (13.6)	9 (23.7)		
	Tongue	13	4(22.2)	9(21.4)			5 (22.7)	8 (21.1)		
	Others	0	0(0)	0(0)			0 (0)	0 (0)		
pT	T1	20	6(33.3)	14(33.3)	2.078	0.556	7 (31.8)	13(34.2)	0.829	0.843
	T2	22	6(33.3)	16(38.1)			8 (36.4)	14(36.8)		
	T3	10	2(11.1)	8(19)			3 (13.6)	7 (18.4)		
	T4	8	4(22.2)	4(9.5)			4 (18.2)	4 (10.5)		
pN	N0	32	10(55.6)	22(52.4)	0.264	0.876	11 (50)	21(55.3)	1.13	0.568
	N1	23	7(38.9)	16(38.1)			10(45.5)	13(34.2)		
	N2	5	1(5.6)	4(9.5)			1 (4.5)	4 (10.5)		
pM	M0	55	17(94.4)	38(90.5)	0.26	0.61	20(90.9)	35(92.1)	0.026	0.872
	M1	5	1(5.6)	4(9.5)			2 (9.1)	3 (7.9)		
Stage	I	13	4(22.2)	9(21.4)	2.254	0.521	5 (22.7)	8 (21.1)	0.375	0.945
	II	17	7(38.9)	10(23.8)			7 (31.8)	10(26.3)		
	III	17	3(16.7)	14(33.3)			6 (27.3)	11(28.9)		
	IV	13	4(22.2)	9(21.4)			4 (18.2)	9 (23.7)		
Recurrence	Absent	56	17(94.4)	39(92.9)	0.051	0.821	20(90.9)	36(94.7)	0.328	0.567
	Present	4	1(5.6)	3(7.1)			2 (9.1)	2 (5.3)		
OS	<36 m	38	8(44.4)	30(71.4)	3.951	**0.047***	12(54.5)	26(68.4)	1.155	0.282
	>36 m	22	10(55.6)	12(28.6)			10(45.5)	12(31.6)		
DFS	<36 m	42	10(55.6)	32(76.2)	2.555	0.11	14(63.6)	28(73.7)	0.67	0.413
	>36 m	18	8(44.4)	10(23.8)			8 (36.4)	10(26.3)		
Deaths	Alive	47	16(88.9)	31(73.8)	1.688	0.194	19(86.4)	28(73.7)	1.32	0.251
	Dead	13	2(11.1)	11(26.2)			3 (13.6)	10(26.3)		

The correlation of TB/HPF and 10HPF (CD44, 40x) using Chi-square test was seen correlated to age, sex, habit, site, tumor stage, recurrence and survival (OS/DFS) but none were found statistically significant. CD44 expression in TB was seen significantly correlated with OS when evaluated in 1HPF ($p = 0.047$).

* P value < 0.05 [LNM-Lymph node metastasis; OS-Overall survival; DFS-Disease free survival]

expression and patients' age, gender, habit, and tumor location. Similar results have been also reported in other studies [2, 27, and 46]. However, CD44 over expression showed predominance in patients aged > 40 years, males population, and those with habit history.

The present result indicates that CD44-overexpression was observed in tumors affecting BM > GBS/RM > Tongue (1HPF:54.8 %>23.8 %> 21.4 %; 10HPF:55.3 %>23.7 %>21.1 %). This was according to the findings by Krump & Ehrmann [25] who reported CD44 expression to be strongly reduced in tongue carcinomas compared with other areas of the oral cavity. Also, this could be due to the unequal distribution of sites in the study sample. In addition, CD44-overexpression was associated with clinically late-stage tumors, mostly stage III (1HPF:33.3 % and 10HPF:28.9 %) although no statistical significance was found in this analysis. This was similar to the studies done by Wang et al. [21], Kokko et al. [22], Mărgăritescu et al. [23], Lee et al. [26] and Saghravanian et al. [27].

Studies have reported that CD44 expression correlated with tumor recurrence and reduced overall survival [27,48]. In this study, the CD44 expression was seen high in non-recurrent cases (1HPF:94.4 % & 10HPF: 94.7 %) and in majority of the recurrent cases (1HPF:7.1 % & 10HPF:5.3 %) which suggests that over expression of CD44 tended to be associated with poor prognosis [Fig. 3]. Also, overexpression of CD44 was associated with lower OS (1HPF:71.4 % & 10HPF:68.4 %) which was statistically significant ($p = 0.047$ in 1HPF). Similarly, CD44 overexpression was associated with reduced DFS (1HPF:76.2 % & 10HPF:73.7 %). Similar findings were observed by Lee et al. [26], Joshua et al. [24] and Kokko et al. [22].

4.2.2. Correlation of TB with histopathologic characteristics using CD44 in 1HPF and 10HPF

[Table 5] According to the results obtained in the current investigation, over expression of CD44 was found in keratinised OSCCs (1HPF:97.6 % & 10HPF:97.4 %) which was statistically significant ($p = 0.037$ in 1HPF). CD44 over expression was observed in cases with abundant NP (1HPF & 10HPF:45.2 %) which was also statistically significant ($p = 0.042$ in 10HPF). Boxberg et al. [28] had shown CD44 over expression having a significant correlation with increased HR. Although CD44 over expression correlated with DK and increased HR (1HPF:40.5 % & 10HPF:44.7 %) in the current analysis, but did not find any statistical significance. In this study, POI type III was associated with CD44 over expression (1HPF:40.5 % & 10HPF:44.7 %) with statistical significance (1HPF: $p = 0.005$ & 10HPF: $p = 0.027$), which indicates WPOI (spray-like) is associated with over expression of CD44. Morand et al. [29] found that tumors with greater DOI showed stronger CD44 expression, suggestive of EMT and CSC signal enabling tumor invasion and metastasis to the nearby lymph node. They suggested CD44 can be used as prognostic predictive marker for DOI and metastatic disease. In the current study, CD44 over expression was noted in tumors with DOI (>4 mm) (1HPF:85.7 % & 10HPF:84.2 %) but was not statistically significant.

In present study, when correlated with tumor grade (Broder's grading), CD44 overexpression was observed in WDSCC>MDSCC>PDSCC (1HPF: 78.6 %>21.4 %>0 & 10HPF: 78.9 %> 21.1 %>0). The difference in the CD44 expression between the groups was found to be statistically significant ($p = 0.037$ in 1HPF). The immunostaining degree is proportional to the grade of differentiation of OSCC. The decrease in the intensity of CD44 expression with the increase in the grade of the tumor suggests reduced cell-to-cell adhesion, resulting in easy detachment of the cells from a rigid constitution. Low expression of CD44 in OSCC tissues may be an indicator of high metastatic potential and maybe related to LNM. So, decreased expression of CD44 may correlate with poor prognosis as suggested by Carnici et al. [49] and Hema et al. [50]. In contrast to our results, studies have demonstrated that CD44 over expression had statistical significant association with higher grades of OSCC [27].

Boxberg et al. [28] correlated PVI, PNI and stromal content with CD44 expression but no statistically significant correlations were obtained. In our study, CD44 over expression was more in cases without PVI (1HPF:81 % & 10HPF:78.9 %) & PNI (1HPF:69 % & 10HPF:68.4 %) although there was no statistical significance. Also, over expression of CD44 was found in cases with higher stromal content (1HPF: 50 % & 10HPF:47.4 %) but did not found any statistical significance.

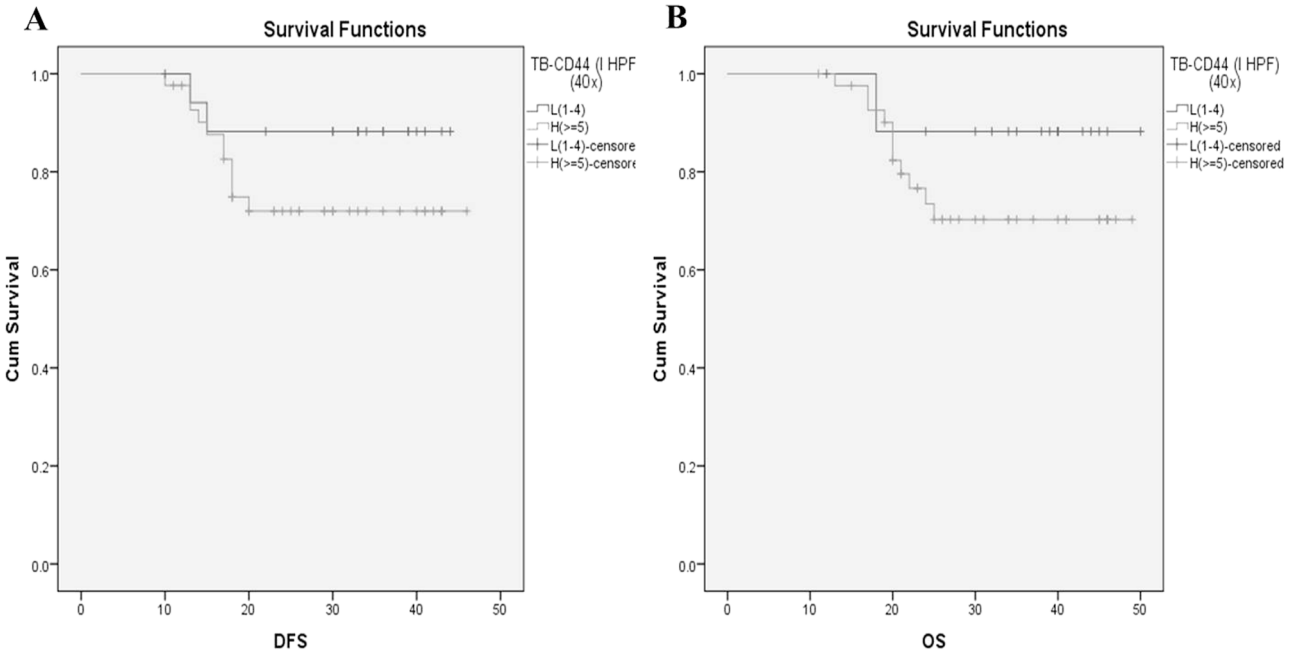

Fig. 3. Correlation of CD44 expression with patient survival outcomes (DFS and OS) using Kaplan-Meier survival analysis: [A] at time zero, the disease free survival probability is 1.0 (or 100 % of the participants are alive). At time > 36 months, the probability of disease free survival is approximately 0.75 (or 75 %) for H (>=5) and 0.85 (or 85 %) for L (1−4) TB-CD44 (1HPF, 40x). [B] At time zero, the overall survival probability is 1.0 (or 100 % of the participants are alive). At time > 36 months, the probability of overall survival is approximately 0.70 (or 70 %) for H (>=5) and 0.89 (or 89 %) for L (1−4) TB-CD44 (1HPF 40x).

Table 5
Correlation of TB according to Boxberg's criteria (1HPF and 10HPFs, 40x) using CD44 expression with histopathological parameters.

Parameter	Categories	N	TB/HPF (CD44,40x)				TB/10HPF (CD44,40x)			
			Low N (%)	High N (%)	Chi square	P value	Low N (%)	High N (%)	Chi square	P value
Keratinization	NK	1	0(0)	1(2.4)	6.607	**0.037***	0 (0)	1 (2.6)	4.665	0.097
	K	59	18(100)	41(97.6)			22 (100)	37 (97.4)		
DK	High	19	8(44.4)	11(26.2)	2.722	0.437	10 (45.5)	9 (23.7)	3.956	0.266
	Moderate	21	6(33.3)	15(35.7)			7 (31.8)	14 (36.8)		
	Minimal	18	4(22.2)	14(33.3)			5 (22.7)	13 (34.2)		
	Absent	2	0(0)	2(4.8)			0 (0)	2 (5.3)		
NP	Little	7	4(22.2)	3(7.1)	4.903	0.179	4(22.2)	3(7.1)	8.216	**0.042***
	Moderately-abundant	29	10(55.6)	19(45.2)			10(55.6)	19(45.2)		
	Abundant	23	4(22.2)	19(45.2)			3(16.7)	20(47.6)		
	Extreme	1	0(0)	1(2.4)			1(5.6)	0(0)		
HR	Marked	13	1(5.6)	12(28.6)	4.911	0.086	3 (13.6)	10 (26.3)	1.349	0.509
	Moderate	29	12(66.7)	17(40.5)			12 (54.5)	17 (44.7)		
	Slight	18	5(27.8)	13(31)			7 (31.8)	11 (28.9)		
POI	Closed	7	2(11.1)	5(11.9)	10.716	**0.005***	4 (18.2)	3 (7.9)	7.23	**0.027***
	Finger-like	32	15(83.3)	17(40.5)			15 (68.2)	17 (44.7)		
	Spray-like	21	1(5.6)	20(47.6)			3 (13.6)	18 (47.4)		
DOI	<4 mm	9	3(16.7)	6(14.3)	0.056	0.813	3 (13.6)	6 (15.8)	0.051	0.822
	>4 mm	51	15(83.3)	36(85.7)			19 (86.4)	32 (84.2)		
Grade	Well-diff	48	15(83.3)	33(78.6)	6.607	**0.037***	18 (81.8)	30 (78.9)	4.665	0.097
	Mod-diff	10	1(5.6)	9(21.4)			2 (9.1)	8 (21.1)		
	Poorly-diff	2	2(11.1)	0(0)			2 (9.1)	0 (0)		
PVI	Absent	49	15(83.3)	34(81)	0.048	0.827	19 (86.4)	30 (78.9)	0.512	0.474
	Present	11	3(16.7)	8(19)			3 (13.6)	8 (21.1)		
PNI	Absent	42	13(72.2)	29(69)	0.06	0.806	16 (72.7)	26 (68.4)	0.123	0.726
	Present	18	5(83.3)	13(31)			6(27.3)	12 (31.6)		
Stromal content	Very low	10	5(27.8)	5(11.9)	3.016	0.389	6 (27.3)	4 (10.5)	3.158	0.368
	Low	18	6(33.3)	12(28.6)			6 (27.3)	12 (31.6)		
	Moderate	27	6(33.3)	21(50)			9 (40.9)	18 (47.4)		
	High	5	1(5.6)	4(9.5)			1 (4.5)	4 (10.5)		

CD44 expression was found significantly correlated with keratinizing tumors, WPOI, and tumor grade when assessed using CD44 expression (1HPF,40x) and with nuclear pleomorphism, WPOI when assessed using CD44 expression (10HPF,40x). Though, most of the tumors exhibited high TB with increased DK, DOI, HR, PVI, PNI, and stromal content, none of them showed any statistical significance.

* P value < 0.05 [NK-Non-keratinized; K-Keratinized; DK-Degree of keratinization; NP-Nuclear pleomorphism; HR-Host response; POI-Pattern of invasion; DOI-Depth of invasion; PVI-Perivascular invasion; PNI-Perineural invasion]

5. Conclusion

The present study makes an attempt to evaluate the association among TB and CD44 expression with the clinicopathologic and histomorphologic parameters. The results of the study suggest varied expression of CD44 in the OSCC cases, but it can be said that the reduced expression of CD44 even in high-grade OSCC samples may be an indicator of high metastatic potential. That is, the down regulation of CD44 could pave way for the cells to detach and invade. Thus, it can be inferred that the loss of cell-adhesion in poorly differentiated tumors correlated to the decreased of CD44 expression might be of prognostic value in the evolution of OSCC. Survival analysis revealed both HTB and CD44 over expression were associated with poor survival showing CD44 over expression as a useful indicator for predicting the prognosis of patients with OSCC. Nevertheless, the significance of CD44 expression in the diagnosis and treatment of OSCC requires further investigation.

Statement of ethics

This research complied with the guidelines for human studies and includes evidence that the research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The Subjects in this study doesn't require any informed consent as this is a retrospective study. This study protocol was approved by the Institutional Review Board, SDM College of Dental Sciences and Hospital, Shri Dharmasthala Manjunatheshwara University (IRB. No.2018/P/OP/59).

Funding

None.

Author statement

The publication of this paper was supported by all the authors, and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere including electronically in the same form, in English or in any other language, without the written consent of the copyright-holder.

Declaration of Competing Interest

There is no conflict of interest.

Acknowledgments

The authors thank Prof. Balram Naik, Principal, Dr. Kaveri Kallikeri, Professor and Head, Department of Oral Pathology, and Department of Oral Surgery, SDM College of Dental Sciences and Hospital, Shri Dharmasthala Manjunatheshwara University for their support and assistance throughout the study. Authors would also like to acknowledge Mrs. Hemalata Gudagudi for her technical assistance and Biogenex Life-sciences limited (California, USA) for providing CD44 antibody with secondary Kit.

References

[1] K. Kaveh, L. Nathan, L. Reigh-Yi, The role of cancer stem cells in head and neck squamous cell carcinoma and its clinical implications, in: E. Dmitry Bulgin (Ed.), New Aspects in Molecular and Cellular Mechanisms of Human Carcinogenesis, InTech, Saint Louis, MO, 2016, pp. 97–113.

[2] M.A. Gonzalez-Moles, C. Scully, I. Ruiz-Avila, J.J. Plaza-Campillo, The cancer stem cell hypothesis applied to oral carcinoma, Oral. Oncol. 49 (2013) 738–746.

[3] M. Boxberg, C. Götz, S. Haidari, C. Dorfner, M. Jesinghaus, E. Drecoll, et al., Immunohistochemical expression of CD 44 in oral squamous cell carcinoma in relation to histomorphologic parameters and clinicopathological factors, Histopathology (2018) 1–14.
[4] P.V. Angadi, P.V. Patil, K. Hallikeri, M.D. Mallapur, S. Hallikerimath, A.D. Kale, Tumor budding is an independent prognostic factor for prediction of lymph node metastasis in oral squamous cell carcinoma, Int. J. Surg. Pathol. 23 (2015) 102–110.
[5] T. Shpitzer, Y. Hamzany, G. Bahar, R. Feinmesser, D. Savulescu, I. Borovoi, et al., Salivary analysis of oral cancer biomarkers, Br. J. Cancer 101 (2009) 1194–1198.
[6] M. Boxberg, M. Jesinghaus, C. Dorfner, C. Mogler, E. Drecoll, A. Warth, et al., Tumour budding activity and cell nest size determine patient outcome in oral squamous cell carcinoma: proposal for an adjusted grading system, Histopathology 70 (2017) 1125–1137.
[7] A. Almangush, T. Salo, J. Hagstrom, I. Leivo, Tumor budding in head and neck squamous cell carcinoma- a systematic review, Histopathology 65 (2014) 587–594.
[8] T. Imai, The growth of human carcinoma: a morphological analysis, Fukuoka Igaku Zasshi 45 (1954) 13–43.
[9] T. Morodomi, H. Isomoto, K. Shirouzu, K. Kakegawa, K. Irie, M. Morimatsu, An index for estimating the probability of lymph node metastasis in rectal cancers, Lymph. node Metastas. Histopathol. Act. Invasive Reg. Cancer Cancer 63 (1989) 539–543.
[10] W.R. Luo, F. Gao, S.Y. Li, K.T. Yao, Tumour budding and the expression of cancer stem cell marker aldehyde dehydrogenase 1 in nasopharyngeal carcinoma, Histopathology 61 (2012) 1072–1081.
[11] A. Almangush, I.O. Bello, H. Keski-Santti, L.K. Makinen, J.H. Kauppila, M. Pukkila, et al., Depth of invasion, tumor budding, and worst pattern of invasion: prognostic indicators in early-stage oral tongue cancer, Head. Neck 36 (2013) 811–818.
[12] C. Wang, H. Huang, Z. Huang, et al., Tumor budding correlates with poor prognosis and epithelial–mesenchymal transition in tongue squamous cell carcinoma, J. Oral. Pathol. Med. 40 (2011) 545–551.
[13] Z. Zhang, M.S. Filho, J.E. Nor, The biology of head and neck cancer stem cells, Oral. Oncol. 48 (2012) 1–9.
[14] S. Krishnamurthy, J.E. Nor, Head and neck cancer stem cells, J. Dent. Res. 91 (2012) 334–340.
[15] L.T. Senbanjo, M.A. Chellaiah, CD44: a multifunctional cell surface adhesion receptor is a regulator of progression and metastasis of cancer cells, Front Cell Dev. Biol. 5 (2017) 18.
[16] N.S. Basakran, CD44 as a potential diagnostic tumor marker, Saudi Med. J. 36 (2015) 273–279.
[17] A. Almangush, M. Pirinen, I. Heikkinen, A.A. Mäkitie, T. Salo, I. Leivo, Tumour budding in oral squamous cell carcinoma: a meta-analysis, Br. J. Cancer 118 (2017) 557.
[18] A.D. Grigore, M.K. Jolly, D. Jia, M.C. Farach -Carson, H. Levine, Tumor budding: the name is EMT. Partial EMT, J. Clin. Med. 5 (2016) 51.
[19] T. Kirita, N. Yamakawa, N. Ueda, T. Yagyuu, Tumor budding as a useful prognostic indicator in early oral squamous cell carcinoma, J. Cancer Sci. Ther. 10 (2018) 162–167.
[20] B. Mack, O. Gires, CD44s and CD44v6 expression in head and neck epithelia, Plos One 3 (2008), e3360.
[21] S.J. Wang, G. Wong, A.M. de Heer, W. Xia, L.Y. Bourguignon, CD44 variant isoforms in head and neck squamous cell carcinoma progression, Laryngoscope 119 (2009) 1518–1530.
[22] L.L. Kokko, S. Hurme, S.M. Maula, K. Alanen, R. Grénman, I. Kinnunen, et al., Significance of site-specific prognosis of cancer stem cell marker CD44 in head and neck squamous-cell carcinoma, Oral. Oncol. 47 (2011) 510–516.
[23] C. Mărgăritescu, D. Pirici, C. Simionescu, A. Stepan, The utility of CD44, CD117 and CD133 in identification of cancer stem cells in oral squamous cell carcinomas, Rom. J. Morphol. Embryol. 52 (2011) 985–993.
[24] B. Joshua, M.J. Kaplan, I. Doweck, R. Pai, I.L. Weissman, M.E. Prince, et al., Frequency of cells expressing CD44, a head and neck cancer stem cell marker: correlation with tumor aggressiveness, Head. Neck 34 (2012) 42–49.
[25] M. Krump, J. Ehrmann, Differences in CD44s expression in HNSCC tumours of different areas within the oral cavity, Biomed. Pap. Med. Fac. Univ. Palacky. Olomouc Czech Repub. 157 (2013) 280–283.
[26] H.J. Lee, Y.H. Kang, J.S. Lee, J.H. Byun, U.K. Kim, S.J. Jang, et al., Positive expression of NANOG, mutant p53, and CD44 is directly associated with clinicopathological features and poor prognosis of oral squamous cell carcinoma, BMC Oral. Health 15 (2015) 153.
[27] N. Saghravanian, K. Anvari, N. Ghazi, B. Memar, M. Shahsavari, M.A. Aghaee, Expression of p63 and CD44 in oral squamous cell carcinoma and correlation with clinicopathological parameters, Arch. Oral. Biol. 82 (2017) 160–165.
[28] M. Boxberg, C. Götz, S. Haidari, C. Dorfner, M. Jesinghaus, E. Drecoll, et al., Immunohistochemical expression of CD 44 in oral squamous cell carcinoma in relation to histomorphologic parameters and clinicopathological factors, Histopathology 73 (2018) 559–572.
[29] G.B. Morand, K. Ikenberg, D.G. Vital, et al., Preoperative assessment of CD44-mediated depth of invasion as predictor of occult metastases in early oral squamous cell carcinoma, Head. Neck 41 (2019) 950–958.
[30] W. Remmele, H.E. Stegner, Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue, Pathologe 8 (1987) 138–140.
[31] M. Bryne, H.S. Koppang, R. Lilleng, A. Kjaerheim, Malignancy grading of the deep invasive margins of oral squamous cell carcinomas has high prognostic value, J. Pathol. 166 (1992) 375–381.
[32] M. Brandwein -Gensler, M.S. Teixeira, C.M. Lewis, et al., Oral squamous cell carcinoma: histologic risk assessment, but not margin status, is strongly predictive of local disease -free and overall survival, Am. J. Surg. Pathol. 29 (2005) 167–178.
[33] A. Lugli, R. Kirsch, Y. Ajioka, et al., Recommendations for reporting tumor budding in colorectal cancer based on the international tumor budding consensus conference (ITBCC) 2016, Mod. Pathol. 30 (2017) 1299–1311.
[34] A. Almangush, M. Karhunen, S. Hautaniemi, T. Salo, I. Leivo, Prognostic value of tumour budding in oesophageal cancer: a meta-analysis, Histopathology 68 (2016) 173–182.
[35] E. Karamitopoulou, Tumor budding cells, cancer stem cells and epithelial-mesenchymal transition-type cells in pancreatic cancer, Front Oncol. 2 (2013) 209.
[36] F.J. Gujam, D.C. McMillan, Z.M. Mohammed, J. Edwards, J.J. Going, The relationship between tumour budding, the tumour microenvironment and survival in patients with invasive ductal breast cancer, Br. J. Cancer 113 (2015) 1066–1074.
[37] K. Kadota, J. Nitadori, K.M. Woo, C.S. Sima, D.J. Finley, V.W. Rusch, P. S. Adusumilli, W.D. Travis, Comprehensive pathological analyses in lung squamous cell carcinoma: single cell invasion, nuclear diameter, and tumor budding are independent prognostic factors for worse outcomes, J. Thorac. Oncol. 9 (2014) 1126–1139.
[38] Y. Yamaguchi, G. Ishii, M. Kojima, K. Yoh, H. Otsuka, Y. Otaki, et al., Histopathologic features of the tumor budding in adenocarcinoma of the lung: tumor budding as an index to predict the potential aggressiveness, J. Thorac. Oncol. 5 (2010) 1361–1368.
[39] N. Xie, C. Wang, X. Liu, R. Li, J. Hou, X. Chen, H. Huang, Tumor budding correlates with occult cervical lymph node metastasis and poor prognosis in clinical early-stage tongue squamous cell carcinoma, J. Oral. Pathol. Med. 44 (2015) 266–272.
[40] C.G. Attramadal, S. Kumar, M.E. Boysen, H.P. Dhakal, J.M. Nesland, M. Bryne, Tumor budding, EMT and cancer stem cells in T1-2/N0 oral squamous cell carcinomas, Anticancer Res 35 (2015) 6111–6120.
[41] I. Zlobec, A. Lugli, Epithelial mesenchymal transition and tumor budding in aggressive colorectal cancer: tumor budding as oncotarget, Oncotarget 1 (2010) 651–661.
[42] S. Acharya, M. Raj, K. Hallikeri, A. Desai, Histological assessment of budding and depth of invasion model in biopsies of oral squamous cell carcinoma, J. Oral. Maxillofac. Pathol. 24 (2020) 581.
[43] A. Elseragy, T. Salo, R.D. Coletta, et al., A proposal to revise the histo-pathologic grading system of early oral tongue cancer incorporating tumor budding, Am. J. Surg. Pathol. 43 (2019) 703–709.
[44] M. Seki-Soda, T. Sano, H. Koshi, S. Yokoo, T. Oyama, Histopathological changes in tumor budding between biopsy and resected specimens from patients treated with preoperative S-1 chemotherapy for oral cancer, J. Oral. Pathol. Med. 48 (2019) 880–887.
[45] B. Majumdar, S. Patil, S. Sarode, G. Sarode, R.S. Rao, Clinico-pathological prognosticators in oral squamous cell carcinoma: an update, Transl. Res. Oral. Oncol. 2 (2017) 1–14.
[46] M.R. Dourado, K.Y.M. Miwa, G.B. Hamada, L.M.R. Paranaiba, Sawazaki-Calone, C. B. Domingueti, et al., Prognostication for oral squamous cell carcinoma patients based on the tumour–stroma ratio and tumour budding, Histopathology 76 (2020) 906–918.
[47] M. Andratschke, S. Chaubal, C. Pauli, B. Mack, H. Hagedorn, B. Wollenberg, Soluble CD44v6 is not a sensitive tumor marker in patients with head and neck squamous cell cancer, Antican Res. 25 (2005) 2821–2826.
[48] K. Chikimatsu, G. Takahashi, K. Sakakura, S. Ferrone, K. Masuyama, Immunoregulatory properties of CD44+ cancer stem-like cells in squamous cell carcinoma of the head and neck, Head. Neck 33 (2011) 208–215.
[49] F. Carnici, G. Stabellini, M. Calvitti, S. Pelucchi, L. Targa, A. Farina, et al., CD44 as a prognostic factor in oral and oropharyngeal factor squamous cell carcinoma, J. Craniofac Surg. 13 (2002) 85–90.
[50] K.N. Hema, K. Rao, H.U. Devi, N.S. Priya, T. Smitha, H.S. Sheethal, Immunohistochemical study of CD44s expression in oral squamous cell carcinoma-its correlation with prognostic parameters, J. Oral. Maxillofac. Pathol. 18 (2014) 162–168.