Original Research

Risk stratification of submandibular salivary gland involvement in oral squamous cell carcinoma based on histopathological parameters: A 15-year retrospective study

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Abstract

Objective: Squamous cell carcinoma (SCC) represents about 90% of all oral malignancies. The study aimed to assess the involvement of the submandibular salivary gland (SMG) in oral SCC (OSCC) patients and the need for SMG excision.

Materials and Methods: Demographics, clinical information and staging of the 210 patients undergoing surgery for OSCC were obtained from the department records. The histopathological slides were retrospectively reviewed. The nodal status was also verified with the histopathology reports. Frequency distribution, Chi-square association, ordinal logistic regression analysis and Kaplan–Meier analysis were performed.

Results: SMG was excised in 171 patients. Five patients had SMG involvement. Buccal mucosa (BM) and gingivobuccal sulcus had a greater risk of level IB metastases ($P < 0.01$). Pattern 3 and pattern 4 of invasion had a higher risk of level IB metastases ($P = 0.04$). Depth of invasion (DOI) >4 mm was associated with level IB lymph node (LN) involvement ($P = 0.0001$). DOI >4 mm to 8 mm had 3.7 times the risk and a DOI >8 mm to 12 mm had 5 times the risk of level IB metastases. Pattern of invasion (POI), tumour budding and DOI >4 mm were significant prognosticators for patient survival.

Conclusion: Histologically, patients may be categorised as 'high risk': those with an increased risk of level IB LN involvement and 'low risk': those at low risk for level IB involvement with the help of POI, tumour budding and DOI as risk factors. In low-risk patients, SMG may be spared and the level IB LNs are dissected. High-risk patients may be chosen as candidates for SMG transfer or excision based on the extent of LN involvement.

Keywords: IB metastases, OSCC, submandibular gland excision

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INTRODUCTION

Squamous cell carcinoma (SCC) is the most common malignancy affecting the oral cavity and represents about 90% of all oral malignancies.[1] There are 300,000 new cases of oral SCC (OSCC) per year with 145,000 deaths per year.[2] India has the highest incidence and prevalence of OSCC (20% of oral cancers worldwide).[2] The patients present either at an early stage (stage I and II) or with an advanced disease stage (stage III and IV). Advanced-stage patients are treated radically, with a multimodal approach, and early-stage patients are usually treated with surgical resection, along with neck dissection, with or without postoperative radiation therapy. Patients with clinical or radiological evidence of cervical lymph node (LN) metastasis undergo therapeutic neck dissection along with excision of the primary lesion. Patients with stage I or II disease often undergo elective neck dissection. Although this has been often debated, studies have found that elective neck dissection has improved overall survival by 12.5% in these patients.[3]

The traditional neck dissection technique (modified and radical) involves the excision of submandibular salivary gland (SMG), despite the low rate of metastases to the gland owing to its anatomical proximity to level IB LNs.[4-7] Surgical management and postoperative radiation greatly compromise the quantity and composition of salivary flow,[8,9] leading to difficulty in swallowing, speaking, chewing and taste perception. This leads to poor food consumption and weight loss and greatly affects the psychology of the patient who is already burdened with the disease.[10,11] The parotid gland is most often affected by radiation in these patients; if the SMG, which secretes 70–80% of the unstimulated saliva, is also excised, these problems worsen and greatly affect their quality of life. Therefore, the importance of salvaging the SMG in these patients becomes important for a better quality of life for the patient.

Sparing the SMG has been a topic of debate for a long time, with no consensus. A study by the American Head and Neck Society has stated that 43.1% of surgeons try to preserve the SMG during the dissection of level IB LNs.[12] This being the scenario, occult metastases of level IB LNs in 27% of the cases of OSCC have also been reported. There are very few studies analysing the frequency of SMG involvement by OSCC and exploring the predictors[13] of level IB metastases, which most often is the determining factor for SMG excision owing to its anatomical proximity.

Previous studies have addressed the involvement of the SMG by OSCC, without assessing risk factors for level IB LN involvement, which is in anatomical proximity to the gland. This study is designed to retrospectively study the frequency of SMG involvement and level IB LN involvement in OSCC patients, to further analyse whether the clinical features and histopathological parameters can predict level IB LN metastasis and a possibility to identify such patients of low risk and salvage the SMG in these patients.

MATERIALS AND METHODS

Case selection

A total of 210 cases were included in the study. Only patients with complete clinical details and slides with the availability of sufficient tumour material for histopathological evaluation were included. Verrucous carcinoma, early SCC (carcinoma *in situ*) and microinvasion (superficial invasion <2 mm and within the lamina propria) cases were excluded. The demographics, clinical information and staging of the patients undergoing surgery for OSCC were obtained from the department records. The histopathological slides were retrospectively reviewed. The study was approved by the Scientific Review Board of Saveetha Dental College, Saveetha University.

Histopathological assessment

The slides were evaluated on the following histopathological parameters: grade of the tumour, tumour budding, depth of invasion (DOI), shape of the tumour nest, lymphoid response at the tumour–host interface, pattern of invasion (POI), lymphovascular invasion (LVI) and perineural invasion (PNI) as per the criteria proposed by Brandwein Gensler.[14] The criteria for histopathological evaluation are summarised in Table 1. These parameters give us a perspective on the patient's propensity for locoregional recurrence and overall survival. The nodal status and submandibular gland involvement were noted from the histopathological assessment of the slides and verified with the histopathology reports.

Statistical analysis

The data were analysed using Statistical Package for Social Sciences (SPSS) version 23.0. The frequency distribution of all the independent variables was analysed using descriptive analysis. The Chi-square association was performed to find the significant association of all independent variables. Ordinal logistic regression analysis was performed on the significant variables to analyse their potential as a risk factor for level IB LN infiltration. The test is highly sensitive and has better prediction power for risk factor analysis. Multicollinearity between independent variables was tested using a linear regression multicollinearity test (variance

Table 1: Criteria for the evaluation of histopathological parameters

Histopathological parameters	Subtype	Criteria
Grade of the tumour	Well differentiated	Abundant keratin pearl formation. Cells with eosinophilic cytoplasm and prominent intercellular bridges Minimal mitosis
	Moderately differentiated	In between well and poor, individual cell keratinisation attempting keratin pearl formation
	Poorly differentiated	Minimal keratinisation, marked pleomorphism nuclear atypia, abundant mitoses (>20/HPF)
Tumour budding		Isolated tumour cell or clusters of tumour cells (<5 cells) in the invasive front
	Low	<5 tumour buds in a single high-power field
	High	>5 tumour buds in a single HPF
Depth of invasion		Depth of invasion <4 mm, 4–8 mm and >8 mm
Shape of tumour nest	Type A	Tumours >80% of oval or sheet-like cells with a smooth border
	Type B	Tumours with 20% scattered tumour nests or tumour nests with irregular margins
Lymphoid response	Pattern 1	Continuous band of lymphocytic response at the tumour CT interface
	Pattern 2	Dense band of lymphoid infiltrate. The inflammation was discontinuous along the tumour CT interface
	Pattern 3	Limited response with no evidence of lymphoid patch or no lymphoid response
Pattern of invasion	Type 1	Tumour invasion in a broad pushing manner with a smooth outline
	Type 2	Tumour invasion with broad pushing 'fingers', or separate large tumour islands, with a stellate appearance
	Type 3	Invasive islands of the tumour >15 cells per island
	Type 4	Invasive tumour islands <15 cells per island. This includes cord-like and single-cell invasion
Lymphovascular invasion		Nests of tumour cells seen within the Lymphatic or blood vessels
Perineural invasion		Tumour cells surrounding or present within a nerve

inflation factor (VIF) ranged from 1 to 10). Model fit was tested using a full likelihood ratio, which showed a good model fit. Ordinal logistic regression analysis was performed by writing syntax for parameter estimate and exponential β-estimate (odds ratio). Survival analysis was conducted by the Cox regression procedure to find the hazard ratio (HR). A P value < 0.05 was considered significant.

RESULTS

Clinicopathological characteristics

A total of 210 cases of OSCC were included in the study. The mean age of the patients was 52 years. The demographic characteristics of the patients are summarised in Table 2. The most common site of tumour occurrence was buccal mucosa (BM) (36.2%), followed by mandibular alveolus (25.7%) and lateral border of the tongue (24.3%). Of the 210 patients, 185 patients underwent therapeutic or elective neck dissection. Among these patients, level IB LNs were involved in 41 (22%) patients, level IA in 17 (9%) patients and both levels IA and IB in six (3%) patients and uninvolved in 121 patients. SMG was excised in 171 patients, of which, SMG was involved by the tumour in five cases and uninvolved in 166 patients [Table 3]. Among these, four cases had a direct involvement of the SMG by tumour with no nodal involvement BM and gingivobuccal sulcus had a greater risk of level IB metastases than other sites ($P < 0.01$).

Frequency of histopathological characteristics

72.9% of the cases were well-differentiated SCC (WDSCC), 26.7% of the cases were moderately differentiated SCC (MDSCC) and 0.5% of the cases were poorly differentiated SCC (PDSCC). Type 4 POI was present in 49% of the cases, type 3 in 36.2% of cases and type 2 and type 1 in 11.0% and 3.8% of the cases, respectively. Type B tumour nests were present in 70% and type A in 30% of the cases. Low tumour budding was present in 51.4% of cases and high budding activity in 37.1% of cases, and no budding activity was observed in 11.4% of the cases. Pattern 2 inflammatory response was present in 56.7% of cases, pattern 3 in 23.3% of cases and pattern 1 in 20% of cases. PNI was present in 5.2% of the tumours. SMG

Table 2: Demographic characteristics of the patients

Characteristics	*n*	%	Total (%)
Gender			
Male	152	72.4	210 (100%)
Female	58	27.6	
Site of the lesion			
Maxillary alveolus	11	5.2	210 (100%)
Lateral border of the tongue	51	24.3	
Buccal mucosa	76	36.2	
Gingivobuccal sulcus	4	1.9	
Retromolar and buccal mucosa	3	1.4	
Mandibular alveolus	54	25.7	
Floor of the mouth	7	3.3	
Palate	3	1.4	
Maxillary and mandibular alveolus	1	0.5	

Table 3: Anatomical site of cases with submandibular gland involvement

Case	Site	Nodal involvement or direct extension
Case 1	Buccal mucosa	Direct involvement
Case 2	Mandibular alveolus	Direct involvement
Case 3	Mandibular alveolus	Direct involvement
Case 4	Mandibular alveolus	Direct involvement
Case 5	Maxillary alveolus	Level IA, IB, II and III nodes infiltrated

was uninvolved histologically in 65.2% of the cases, had an inflammatory focus in 13.8% and was infiltrated by a tumour in 2.4% of the cases [Table 4].

Correlation of histopathological features with Level IB LN involvement

Type 3 and 4 POI had a higher risk of level IB metastases ($P = 0.04$). Pattern 4 had a 1.2 times higher risk of LN metastases. DOI >4 mm was associated with level IB LN involvement ($P = 0.0001$). DOI >4 mm to 8 mm had 3.7 times the risk and a DOI >8 mm to 12 mm had five times the risk of level IB metastases. Budding had a 1.4 to 2.2 times increased risk of level IB metastases. The host inflammatory response, PNI and LVI did not have any association with level IB LN metastases [Table 5].

Correlation of risk factors with survival

Among the 210 patients, follow-up details were completely available for 104 patients for survival analysis. Type 4 POI had a higher mortality rate compared with types 2 and 3, with a HR of 0.086 and $P = 0.003$ [Figure 1a]. DOI > 4 mm and high tumour budding were associated with an increased risk of mortality, which was statistically significant (HR = 0.046, $P = 0.004$, and HR = 4.908, $P = 0.047$, respectively) [Figure 1b and c]. There was no significant difference in survival with LVI, PNI and the shape of the tumour nest.

DISCUSSION

The present study is the largest single institutional study assessing the histopathological predictors of level IB metastases in 210 cases of OSCC patients. The POI, DOI and tumour budding were strong histopathological predictors of level IB LN metastases. SMG was mostly uninvolved by tumour in 97.6% of the cases.

Our study findings highlight that the SMG was uninvolved by tumour in 97.6% of the cases. The SMG is most commonly excised for two main reasons: i) the direct involvement of the gland by the tumour, which is found to be significantly low, both from the current study (2.4%) and previous literature, and ii) the involvement of the level IB LN, warranting its dissection by proximity, the SMG. First, addressing the issue of direct tumour involvement of SMG, it can be involved by the tumour in the following three ways:[15] i) direct extension of the tumour into the glandular parenchyma, ii) involvement of level IB LN and iii) intraglandular LN metastases. Direct extension of tumour was seen in 2.4% of our cases, which is slightly

Table 4: Frequency of histopathological characteristics of the cases

Characteristics	*n*	%	Total (%)
Grade of tumour			
WDSCC	153	72.9	210 (100%)
MDSCC	56	26.7	
PDSCC	1	0.5	
Pattern of invasion			
1	8	3.8	210 (100%)
2	23	11.0	
3	76	36.2	
4	103	49.0	
Shape of tumour nest			
A	63	30.0	210 (100%)
B	147	70.0	
Tumour budding			
No	24	11.4	210 (100%)
Low	108	51.4	
High	78	37.1	
Inflammatory response			
1	42	20.0	210 (100%)
2	119	56.7	
3	49	23.3	
Lymph vascular invasion			
Absent	209	99.5	210 (100%)
Present	1	0.5	
Perineural invasion			
Absent	199	94.8	210 (100%)
Present	11	5.2	
Salivary gland status			
Not excised	39	18.6	210 (100%)
Not involved	137	65.2	
Inflammatory foci	29	13.8	
Infiltrated	5	2.4	

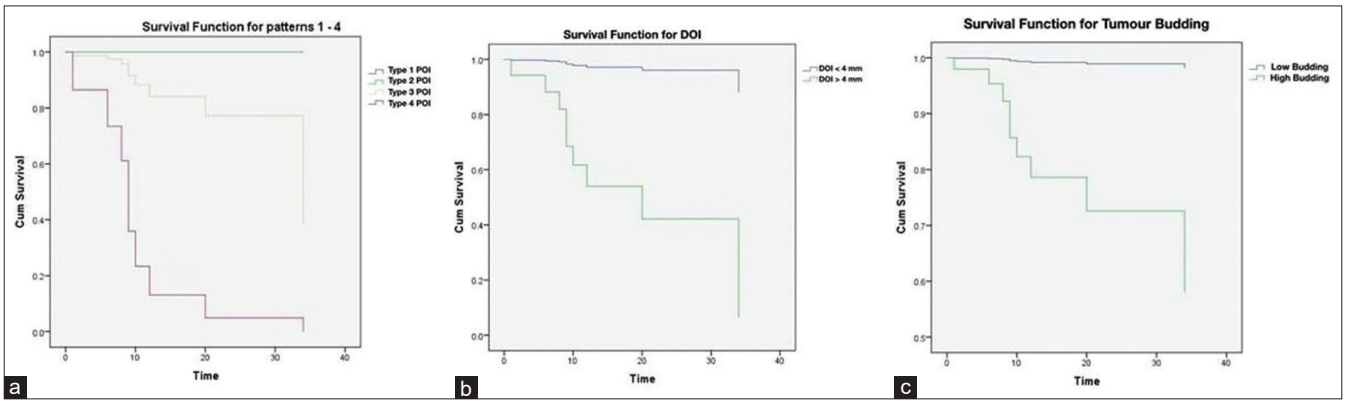

Figure 1: Kaplan–Meier curves for (a) pattern of invasion and survival, (b) depth of invasion and survival and (c) tumour budding and survival

Table 5: Ordinal regression analysis of the significant histological parameters

Characteristics	Model fit (pseudo-R^2) (Nagelkerke R^2)	Exp-β (odds ratio)	*P*	95% confidence interval
Pattern of invasion				
1	24.8%	1	-	-
2		0.384	0.037*	0.155-0.952
3		0.652	0.147	0.365-1.163
4		1.222	0.045*	0.964-1.864
Depth of invasion				
≤4 mm	48.6%	1	-	-
>4 mm–8 mm		3.772	0.000*	2.305-5.236
>8 mm–12 mm		5.068	0.000*	3.081-6.322
Shape of tumour nest				
A	16.8%	1	-	-
B		1.353	0.014*	0.326-1.949
Tumour budding				
No	35.1%	1	-	-
Low		1.473	0.009*	0.697-2.169
High		2.163	0.002*	1.253-2.854
Inflammatory response				
1	29.3%	1	-	-
2		1.148	0.312	0.579-2.208
3		1.513	0.678	0.678-3.376
Perineural invasion				
Absent	14.8%	1	-	-
Present		0.480	0.206	0.153-1.489

higher than the world literature of 1%.[4,16-18] This might be attributed to the anatomical site. Among the five cases, three cases were from the mandibular alveolus, one was from the BM and one patient with SCC of the maxillary alveolus had involvement of level IA, IB, II and III nodes.

Both were locally advanced disease states with anatomical proximity to SMG and had a direct involvement of the SMG by tumour with no nodal involvement. In previous studies by Ebrahim *et al.*, only one of the 107 cases had direct involvement of the SMG.[18] Only two of the 69 cases had direct involvement of the gland by the tumour in the cohort reported by Naidu *et al.*[19] Among the seven cases of SMG metastasis reported by Chen *et al.*,[11] five were by direct invasion of the gland, one by intraglandular nodal involvement and one case by level IB nodal invasion. The site of the tumour is an important predictor for the SMG invasion. In our study, the most commonly involved site was BM (36.2%), mandibular alveolus (25.7%) and tongue (24.3%). Although the tongue is reported as the high-risk site for SMG involvement,[13] BM and alveolus are the most commonly involved sites in the Indian population. These sites have a higher risk of glandular invasion because of their anatomical proximity to the SMG as evident from the results of our study. Due to their anatomical proximity, lesions on the floor of the mouth also seem to have a higher propensity for direct invasion of the SMG, but these lesions were found to be relatively rare in occurrence in the current study (3.3%). LNs are virtually never found in SMG. As LNs are missing within the SMG, interglandular metastases may mostly be LN metastases with direct invasion into the gland.

Next, addressing the other reasons for SMG excision which are- i) to resect involved level IB LNs, ii) to hasten level IB dissection and iii) to obtain adequate clearance of level IB.[17] In most of the circumstances, SMG is excised due to level IB LN involvement. Previous studies have merely identified tumour involvement of SMG without consideration of the level IB LNs, which is in anatomical proximity to the gland and is a major determinant in its excision. To identify patients with a risk of LN metastases that warrant discretion in preserving SMG, the histopathological factors associated with periglandular lymphadenopathy, that is level IB LN metastases, were also assessed in the present study. Among the 210 patients, level IB LNs were involved in 41 patients (19.5%), level IA in 17 patients (8%) and both levels IA and IB in six patients (2%). Histologically, OSCC with POI 4, a DOI >4 mm, a type B tumour nest and budding (low and high) had a high risk of level IB LN metastases. Our present study identified that a patient with POI 4 has 1.2 times the risk of the LN metastases and DOI >4 mm has 3.7 times and DOI >8 mm has five times the risk of level IB LN metastases. Tumour budding has a 1.4 to 2.1 times the increased risk of level IB nodal involvement.

Pathologically, tumour budding is defined as isolated tumour cells or clusters of tumour cells in the invasive tumour front.[20] Tumour budding is significantly associated with an increased risk of LN metastasis.[21-23] A budding score of >5 is usually associated with LN metastases. Corroborating with the underlying pathological mechanisms, tumour budding along with POI is an indicator

of loss of cell adhesion and active tumour invasion and marks the aggressiveness of the tumour.[24] In most cases, the worst POI is associated with tumour budding activity. Metastatic cascade involves the invasion of the stroma by tumour cells, migration into vessels, extravasation of the tumour cells and colonisation of the LNs.[25] Invasion is characterised by the epithelial-to-mesenchymal transition of the tumour cells.[26,27] The correlation between tumour budding and EMT[28] corroborates the fact that budding is an early indicator of tumour metastasis. Also, these are features that can be easily discerned from routine H and E sections.

With regards to resection of level IB LN without excision of SMG, there is no consensus in the scientific literature in this regard. Some authors believe that level IB LNs are situated between the superficial and deep layers of cervical fascia and can be dissected without surgical extraction of the salivary gland.[29] Dhiwakar *et al.*[30] further added that excision of SMG is unnecessary and level IB LNs can be excised without sacrificing the gland. However, Lanzer *et al.*[31] reported that preservation of SMG is not oncologically safe in the floor of the mouth and tongue tumours and was associated with locoregional recurrence. In the Indian subcontinent, most of the patients present with late-stage disease (T3 or T4) and with clinical involvement of the nodes. Macroscopically, we often see the proximity of the level IB LN to the SMG. Hence, the feasibility of preserving the SMG in such instances is debatable. With no previous existing data on the involvement of SMG in BM and Guillain–Barré syndrome (GBS) tumours, the present study identified that SMG is uninvolved in most of the patients with SCC involving the GBS, which are high-risk sites for level IB LN metastases. As it is much debated if periglandular lymphadenopathy allows for a safe SMG-sparing neck dissection, we have also identified histological risk factors for level IB LN metastases. Putting these findings together, we propose the possibility of categorising the patients as 'high risk': those with an increased risk of level IB LN involvement and 'low risk': those at low risk of level IB involvement. In low-risk patients, the SMG may be spared and the level IB LNs are dissected. The high-risk patients may be chosen as candidates for SMG excision based on the extent of LN involvement.

The possibility of translating these results into practice demands a methodologic approach and requires further prospective studies. In this study, the histopathological risk assessment was performed based on the analysis of the excision core samples. Treatment planning is mostly performed following an incisional biopsy. Hence, the feasibility of identifying these three parameters namely POI, tumour budding and DOI needs to be identified. Some authors have identified budding as a good prognostic factor in incisional biopsies.[32] Tumour budding *per se* was an independent prognostic indicator of LN metastases in the present study and those previously reported in the literature.[32-34] Tumour budding is a parameter that is easily discernible with H and E staining and is highlighted better with cytokeratin staining if required and may be used to identify clinically N0 patients at risk of developing LN metastases. Usually, an incisional biopsy is not sufficient to identify the DOI. In such conditions, imaging modalities such as magnetic resonance imaging (MRI) might be used in preoperative clinical workup to identify DOI and categorise these patients. In such cases, ultrasound-guided fine-needle aspiration cytology (FNAC) might be used to identify occult metastases in suspicious nodes. Studies have shown that ultrasound-guided FNAC helps in the detection of occult metastases in OSCC by up to 14%.[35]

Another option would be to identify these parameters in frozen sections to categorise the patients, thereby preserving the SMG.

The present study was the first of its kind in assessing SMG involvement and histopathological risk factors of level IB involvement in a large cohort of 210 patients containing predominantly gingivobuccal sulcus and BM tumours. The study has identified that the direct extension of the tumour is predominantly the only cause of SMG involvement. DOI, type 3 and type 4 POI and tumour budding are prognostic indicators of level IB LN involvement.

Limitations of the study

The present study was a retrospective cross-sectional study. Large prospective studies to identify whether level IB can be dissected without sacrificing the SMG are needed. This would also bring to light the other practical difficulties encountered with large metastatic nodes with proximity or nodes that are matted to the SMG.

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Conflicts of interest

There are no conflicts of interest.

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