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ORIGINAL ARTICLE

Eosinophils may predict occult lymph node metastasis in early oral cancer

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Abstract
Objective The aim of this study was to investigate whether tumor-associated tissue eosinophilia (TATE) in early oral squamous cell carcinoma (OSCC) would aid in predicting occult lymph node metastasis.
Patients and methods Seventy-one patients undergoing elective neck dissection for T1 and T2 OSCC were evaluated for clinical features, prognosis, and TATE. The degree of TATE in OSCC was statistically analyzed in relation to the clinicopathological features, tumor invasion, occult lymph node metastasis, and survival using χ^2 test and Kaplan–Meier method.
Results Statistical analysis revealed that intense TATE was a significant feature (p=0.004) to predict occult lymph node metastasis in patients with early OSCC. All regional recurrences of the OSCC occurred in patients showing intense TATE.
Conclusions These results suggest that intense TATE can be clinically used as a predictive factor for occult lymph node metastasis.
Clinical relevance The presence of intense TATE is an adjunctive histopathological marker to reinforce the indication of elective neck dissection of the patients with early OSCC.

Keywords Oral cancer · Eosinophils · Metastasis

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Introduction

The importance of the lymph node metastasis is well recognized in cancer staging, with lymph node status determining the treatment and prognosis in patients with solid tumors such as head and neck squamous cell carcinoma [1-5]. However, no clinical examination or biochemical markers can be used to assess the metastatic condition of the cervical lymph node accurately [4]. Thus, most centers of treatment favor elective neck dissection in early oral and oropharyngeal squamous cell carcinoma for staging of the neck and removal of occult disease [5].

Although 20%–30% of clinically N0 patients will show occult metastases on elective neck dissection, a considerable number of patients will remain pathologically N0, and therefore, the undesirable cosmetic and functional effects of neck dissection should be avoided [6, 9]. Thus, the problem could be solved if it were possible to predict the risk of neck metastases. However, this type of prediction has been difficult to introduce and apply in clinical practice, and therefore, determining whether or not elective neck dissection will be beneficial to the patients with early oral and oropharyngeal squamous cell carcinoma remains an important clinical dilemma [3].

Many studies in the context of elective neck dissection have investigated the predictive factor for lymph node metastasis in clinically nodal-negative patients with oral cancer [6-9]. Nowadays, there is an urgent need to identify characteristics of the primary tumor that might predict nodal metastasis [7].

In this way, the presence of eosinophils infiltrating in tumor has been associated with locally invasive squamous cell carcinoma, suggesting that the eosinophils may represent an adjunctive feature in the assessment of stromal invasion of head and neck malignant tumor [10-13]. In addition, Falconieri and colleagues [11] showed that oral squamous cell carcinoma (OSCC) rich in eosinophils was associated with metastatic involvement of neck lymph node and concluded that tumoral, rather than local factors, are involved in eosinophilic migration.

Although there is a tendency to consider tumor-associated tissue eosinophilia (TATE) as a favorable prognostic indicator in squamous cell carcinomas [14-17], there are other studies showing its association with a poor prognosis [10, 13, 18], or even with no influence on patient outcome [12, 19, 20].

With this in mind, the purpose of this study was to investigate whether TATE in clinically negative neck (cN0) patients with early OSCC would aid in predicting occult lymph node metastasis.

Patients and methods

Patient and tumor samples

Seventy-one patients undergoing elective neck dissection for T1 and T2 OSCC at the Head and Neck Surgery and Otorhinolaryngology Department of the A.C. Camargo Cancer Hospital, São Paulo, Brazil, from 1977 to 2005 were selected for analyses of the clinical features, prognosis, and the TATE.

The patients were selected following the inclusion criteria: (1) primary OSCC located in the tongue or floor of the mouth, clinical stages I (T1N0M0) and II (T2N0M0), submitted to routine elective neck dissection; (2) patients who did not undergo radiotherapy, chemotherapy, or other treatment prior to surgery; (3) patients without other simultaneous primary tumors; and (4) tumor tissue available for microscopic analysis. Clinical data of the patients were obtained from the medical records and included gender, age, ethnic group (white or not white), tobacco and alcohol consumption, tumor location, TNM stage [21], treatment (surgery and postoperative adjuvant radiotherapy), and clinical follow-up (local recurrence, regional recurrence, and death). This study was approved by the local ethics committee.

TATE analysis

Formalin-fixed 3-μm tumor tissues were taken from archive of the Department of Pathology of A.C. Camargo Cancer Hospital, São Paulo, Brazil, and stained with hematoxylin and eosin for TATE analysis.

Eosinophils at the invasive front of the tumor in each slide in a×400 field were digitally captured using a camera (Axiocam MR3; Zeiss) attached to a light microscope (Axioskop2 Plus; Zeiss, Jena, Germany) and recorded by Axiovision software (Axiovision 4.6; Zeiss). The area of invasive front tumor was totally scanned sequentially, and approximately 15 fields in each tumor were captured for analysis of TATE. The total number of eosinophils divided by the total area was calculated in each tumor sample, and then two degrees of TATE intensity were established based on the median of eosinophils/mm^2 obtained in the 71 OSCCs: (1) absent/mild (0–67eosinophils/mm^2) and (2) intense (68–298 eosinophils/mm^2).

The presence of vascular embolization and perineural, muscular, and salivary gland infiltrations in the OSCC were reviewed in the hematoxylin and eosin-stained tumor sections.

Occult lymph node metastases (pN+) were collected from the hospital records and were categorized as either positive in case of the presence of any cancer deposits (isolated tumor cells and/or micrometastasis and/or metastasis) or as negative if no tumor cells were observed [22].

Statistical analysis

Associations between TATE and clinicopathological variables were verified by the χ^2 test or Fisher's exact test. Survival rates (overall, disease-free, and regional disease-free survival) were calculated by the Kaplan–Meier method, and the comparison of the survival curves was performed using log-rank test. In both tests, p values lower than 0.05 were considered statistically significant.

Results

The associations between TATE and clinicopathological findings of the 71 patients with early OSCCs who underwent surgical treatment and elective neck dissection were summarized in Table 1.

Most patients with OSCC arising from the tongue and floor of the mouth were white men and aged from 35 to 77 years (median, 59 years). Tobacco or alcohol use was frequently associated with the clinical history of OSCC patients presenting tumor with absent/mild or intense TATE (Table 1).

Table 1 Association among TATE, clinicopathological parameters, treatment, and outcome of the 71 patients with oral cancer

Variable	Category	Absent/Mild eosinophilia, n (%)	Intense eosinophilia, n (%)	*p*
Gender	Male	28 (77.8)	27 (77.1)	0.949
	Female	8 (22.2)	8 (22.9)	
Ethnic group	White	31 (86.1)	34 (97.1)	0.199
	Non-white	5 (13.9)	1 (2.9)	
Age	≤59 years	21 (58.3)	18 (51.4)	0.559
	>59 years	15 (41.7)	17 (48.6)	
Tobacco[a]	Yes	26 (78.8)	32 (97.0)	0.054
	No	7 (21.2)	1 (3.0)	
Alcohol[a]	Yes	23 (69.7)	30 (90.9)	**0.030**
	No	10(30.3)	3 (9.1)	
Tobacco+alcohol[a]	Yes	22 (66.7)	30 (90.9)	**0.016**
	No	11 (33.3)	3 (9.1)	
Tumor site	Tongue	20 (55.6)	25 (71.4)	0.165
	Floor of mouth	16 (44.4)	10 (28.6)	
T clinical	T1	9 (25.0)	4 (11.4)	0.139
	T2	27 (75.0)	31 (88.6)	
Local recurrence	Yes	7 (19.4)	5 (14.3)	0.562
	No	29 (80.6)	30 (85.7)	
Regional recurrence	Yes	0 (0)	6 (17.1)	0.055
	No	36 (100)	29(82.9)	
Radiotherapy	Yes	8 (22.2)	12 (34.3)	0.259
	No	28 (77.8)	23 (65.7)	
Second primary tumor	Yes	7 (19.4)	12 (34.3)	0.158
	No	29 (80.6)	23 (65.7)	
Total		36 (100)	35 (100)	

n indicates number of patients; *p* values obtained by χ^2 test or by Fisher's exact test (the bold values indicate statistical significant differences)

[a]Excluded patients with lost records

Tumors showing intense TATE were significantly associated with alcohol consumption (p=0.003) or with a long history of combined ethanol intake and cigarette smoking (p=0.016), as shown in Table 1.

Based on the International Union Against Cancer classification of the oral cavity carcinomas [21], most tumors with absent/mild or intense TATE were classified as T2N0M0 (Table 1). No statistically significant association was detected among TATE and gender, ethnic group, tumor site, and clinical T stage of the patients with OSCC.

All 71 patients underwent to surgical treatment of primary tumor and elective neck dissection: 59 of them were submitted to ipsilateral and 12 to bilateral neck dissection. Twenty OSCC patients received postoperative adjuvant radiotherapy (8 patients with tumors showing absent/mild TATE and 12 patients with tumors presenting intense TATE).

Table 2 Association between TATE and pathological variables of the 71 patients with oral cancer

Variable	Category	Absent/Mild eosinophilia, n (%)	Intense eosinophilia, n (%)	*p*
Lymphatic embolization	Yes	9 (25.0)	14 (40.0)	0.177
	No	27 (75.0)	21 (60.0)	
Blood embolization	Yes	31 (86.1)	30 (85.7)	1.00
	No	5 (13.9)	5 (14.3)	
Perineural infiltration	Yes	16 (55.6)	22 (62.9)	0.120
	No	20 (44.4)	13 (37.1)	
Muscular infiltration	Yes	24 (66.7)	28 (80.0)	0.205
	No	12 (33.3)	7 (20.0)	
Salivary gland infiltration	Yes	12 (33.3)	11 (31.4)	0.864
	No	24 (66.7)	24 (68.6)	
Lymph node status (OLM)	pN0	33 (91.7)	22 (62,9)	**0.004**
	pN+	3 (8.3)	13 (37,1)	
Total		36 (100)	35 (100)	

n indicates number of patients; *p* values obtained by χ^2 test or by Fisher's exact test (the bold values indicate statistical significant differences), OLM, occult

Table 3 Five- and 10-year survival rates of the 71 patients with oral cancer according to the TATE

Tissue eosinophilia	Overall Survival		*p*	Disease-free survival		*p*
	5 years (%)	10 years (%)		5 years (%)	10 years (%)	
Absent; mild	71.0	61.2	0.166	75.9	75.9	0.303
Intense	59.7	46.5		66.4	66.4	

During the clinical follow-up, local recurrence, neck recurrence, and a second primary tumor were detected in OSCC patients, as shown in Table 1. All regional recurrences of the OSCC occurred in patients showing tumors with intense TATE (Table 1). The intensity of TATE was not associated with radiotherapy, local recurrence, or second primary tumor (Table 1)

The relationships between TATE and blood/lymphatic embolization, as well as with perineural, muscular, glandular invasion, were presented in Table 2. There was no significant association between TATE and the variables that were mentioned above.

The log-rank test for regional disease-free survival demonstrated that TATE was highly correlated with regional recurrence (p=0.009). OSCC patients with intense TATE had a significantly higher risk for development of a neck recurrence than those with absent/mild TATE. The 5-year regional disease-free survival rate was 100% for OSCC patients with absent/mild eosinophilia versus 81.8% for the OSCC patients with intense eosinophilia, as shown in Fig. 1.

There were no differences in the 5- and 10-year overall survival and disease-free survival rates between the OSCC patients with absent/mild and intense eosinophilia (Table 3).

Discussion

Elective neck dissection remains the therapeutic management for staging and treatment of the clinically N0 oral cancer patients [2-4, 9, 23].

The indication of neck dissection in oral cancer is a problem of risk–benefit evaluation between the probability of regional metastases, the probability of complications associated with neck dissection, and the possible prognostic influence of late diagnosis of metastasis during follow-up. If the probability of neck metastases is high, to make a neck dissection with its intrinsic morbidity has the same effect as therapeutic dissection, decreasing the risk of regional recurrence. However, if the probability of neck metastases is low or nil, neck dissection simply acts as an overtreatment, where the morbidity of the neck procedure only offers a decrease in quality of life and functional deficits [3].

The frequency of occult lymph node metastases, generally detected by hematoxylin and eosin staining, has been used as a prognostic factor in determining treatment strategies in various patients with oral cancer [2-4, 6, 7]. Therefore, the identification of characteristics of the primary tumor for controlling neck lymph node metastases in these patients is evident [7].

In this context, the present study was performed focusing on the prediction of the risk of occult neck metastases based on the presence of TATE in patients with OSCC.

All 71 patients included in this study were clinically classified as N0, and they underwent a surgical treatment of primary tumor and elective neck dissection. Occult lymph node metastases (pN+) were detected in 22.5% of the OSCC patients, and this frequency is close to those reported by Faustino et al. [8] (22%), Amaral et al. [6] (23%), and Okamoto et al. [24] (24%).

Although the presence of eosinophils infiltrating in tumor, described as TATE, has been demonstrated in oral cancer [11-14, 16, 18, 20], their exact function or their prognostic value in this malignant tumor remains undefined [12, 16, 20].

Our results showed that there was no significant association among TATE and clinical findings, treatment, and outcome, including the following variables: gender, ethnic group, tumor site, T clinical, radiotherapy, local recurrence,

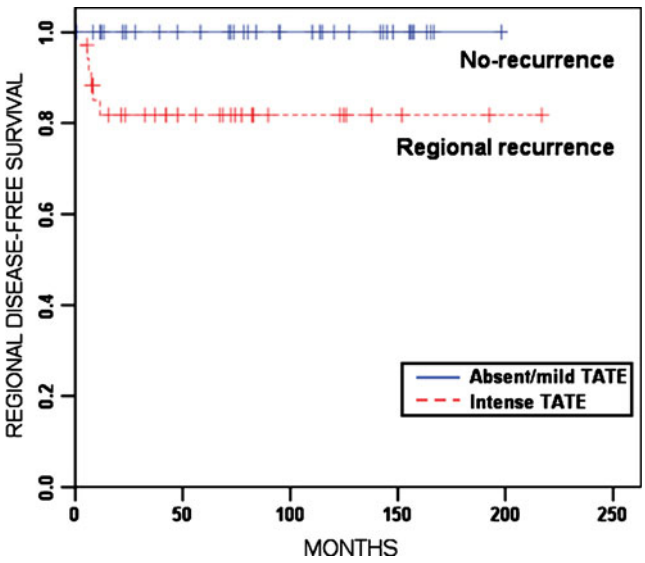

Fig. 1 Cumulative regional disease-free survival probability curves stratified by the TATE. Patients with OSCC and intense TATE had a significantly higher risk for development of a neck recurrence (p=0.009) than those with absent/mild TATE

or second primary tumor. However, tumors showing intense TATE were significantly correlated with alcohol consumption (p=0.003) or with a long history of combined ethanol intake and cigarette smoking (p=0.016), as shown in Table 1.

The present study is the first to have reported the correlation between TATE and occult lymph node metastasis in OSCC. Statistical analysis revealed that intense TATE was significantly (p=0.004) associated with occult lymph node metastasis in patients with early OSCC, as described in Table 2. Although further analyses are required to confirm these results, they corroborate with published studies [10-13] suggesting that a rich eosinophilic stromal infiltrate is invariably associated with invasive carcinoma. Some studies [10, 13] demonstrated that an elevated eosinophil count in the squamous neoplasia of larynx or aerodigestive tract may be a morphologic marker for assessing tumor invasion as well a clinical predictor for aggressive tumor biology. Similar results were observed in OSCC [12], where intense TATE was associated with stromal invasion and correlated with advanced clinical stage of the tumors. Furthermore, the intense presence of eosinophils, as shown in our study, seems to indicate metastatic lymph node involvement of the early oral tumor in patients clinically classified as N0.

Regarding the prognosis of the OSCC, our results did not reveal any significant association between TATE and patient's prognosis, reinforcing previous reports [12, 19, 20] and contrasting with others [13, 14, 16, 18]. There were no significant differences in the 5- and 10-year overall and disease-free survival rates according to TATE (Table 3); however, OSCC patients with intense TATE presented a lower cumulative disease-free survival. This finding indicates a great number of patients whose oral cancer recurred before 60 months of follow-up, demonstrating that OSCC patients presenting intense TATE had poor prognosis as compared with those with absent/mild TATE (Table 3).

Additionally, the regional disease-free survival demonstrated that TATE was highly correlated with regional recurrence (p=0.009). OSCC patient with intense TATE had a significantly higher risk for development of a neck recurrence than those with absent/mild TATE. The 5-year regional disease-free survival rate was 100% for OSCC patients with absent/mild eosinophilia versus 81.8% to the OSCC patients with intense eosinophilia, as shown in Fig. 1. Therefore, we had few patients with oral cancer that presented regional recurrences, and further studies will be required to confirm these findings.

In summary, our results support the evidence suggesting that intense eosinophilic infiltrate in OSCC is an adjunctive microscopic feature to predict occult lymph node metastasis. Eosinophils can be easily recognized in routinely stained tumor sections; then, measurement or identification of intense TATE could become, in the future, useful in reinforcing the indication of elective neck dissection in clinically N0 patients with oral cancer.

Conclusion

The findings suggest that intense TATE is a contributing factor to predict occult lymph node metastasis in patients with early OSCC. In addition, an intense TATE in the invasive front tumor indicates a higher probability of regional recurrence during the follow-up of the same patients. Therefore, the intense TATE can be used as a predictive factor for occult lymph node metastasis and an adjunctive histopathological marker to reinforce the indication of elective neck dissection of the patients with early OSCC clinically N0.

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Conflict of interest The authors have no conflict of interests.

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