Research Article

Tumor-associated Tissue and Blood Eosinophils in Oral Squamous Cell Carcinoma

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Abstract

Purpose: To assess the correlation of Tumor-Associated Tissue Eosinophilia (TATE) and blood eosinophil counts with tumor grade, size, and local lymph node involvement in oral squamous cell carcinoma.

Methods: In the present study, forty-three patients with a diagnosis of oral Squamous Cell Carcinoma (SCC) were evaluated. TATE was determined in ten random fields under ×40 magnification. Laboratory tests of the patients were also evaluated before surgery and repeated at three, six and twelve weeks after surgery. Data were analyzed by descriptive statistical methods (mean ± standard deviation), N (%) , repeated measures ANOVA, Pearson's correlation coefficient and chi-square test. Statistical significance was defined at p<0.05.

Results: There was a correlation between TATE and tumor grade (p=0.03), but there wasn't any correlation between TATE and tumor size or lymph node involvement. In the analysis of tumor-associated blood eosinophil counts, a correlation was observed with lymph node involvement (p= 0.02), but no correlation was noted with the tumor grade or size. In addition, peripheral blood analysis revealed that blood eosinophilia grades were significantly different one day before surgery from 6 and 12 weeks after surgery.

Conclusion: TATE was found to be associated with tumor grade , but not with tumor size or lymph node involvement. A correlation was observed with lymph node involvement, but no correlation was noted with the tumor grade or size. In addition, blood eosinophilia grades were significantly different one day before surgery from 6 and 12 weeks after surgery.

Introduction

Eosinophils were recognized more than one hundred years ago; they were first described by Wharton Jones [1] in 1846 as "coarse granule cells" and later were called eosinophils by Ehrlich [2]. They are myeloid-derived hematopoietic elements that belong to the basophil-eosinophil granulocyte lineage [3], comprising 1-5% of leukocytes in the peripheral blood with an upper limit of 0.4×10^9 per liter [4].
Apart Eosinophils are considered destructive effector leukocytes with cytotoxic activities and are mainly engaged in cases of parasitic infections (e.g. helminthic infections) and allergic diseases (e.g. bronchial asthma, allergic dermatitis etc.). In addition, studies have demonstrated their involvement in tissue remodeling and in innate and acquired immunity response modulation [5].
Eosinophil counts undergo changes by tumor effect. These changes can be found in the peripheral blood count or as tumor-associated tissue eosinophilia (TATE).

TATE is characterized by the presence of eosinophils as a component of peri- and intra-tumoral inflammatory infiltrate [6], which has been reported in squamous cell carcinomas of the oral cavity and cervix [7], breast adenocarcinoma [8,9], large cell carcinoma of lungs [10], and colorectal carcinomas [11].
Prognostic value of eosinophilia is discussed mostly in clinical settings. In the head and neck region, TATE has given rise to a great deal of controversy. Some studies have reported that TATE is correlated with better prognosis [6,12-14]. Other studies, however, have reported that eosinophils may have a role in tumor growth, with a poor prognosis [15-17] or even with no effect on the prognosis [18-20].
The most common cancer in the head and neck region is squamous cell carcinoma and the majority of these cancers occur in the oral cavity [21]. Prognosis in the oral squamous cell carcinoma (OSCC) depends on tumor, treatment and patient factors.
Tumor-related factors are tumor size, anatomic location, lymph

node involvement, degree of differentiation, and tumor cell behavior [22].

The exact role of eosinophils in tumors and peripheral blood is unclear. Since squamous cell carcinoma (SCC) is the most common cancer of the oral cavity, in the present study the possible role of TATE and blood eosinophil counts as a prognostic factor in OSCC was evaluated with respect to tumor size, tumor grade, and local lymph node involvement.

Methods

In a retrospective descriptive study, 43 patients who had previously undergone radical neck dissection surgery for primary oral SCC were randomly selected. These patients were referred to Head and Neck Surgery or Otorhinolaryngology Departments of Imam Khomeini and Imam Reza hospitals of Tabriz University of Medical Sciences from January 1999 to March 2009.

The protocol of the present study was approved by the Research and Ethics Committees of Tabriz University of Medical Sciences.

The inclusion criteria were the following:
1. A history of surgery of the initial treatment followed or not followed by radiotherapy
2. A written record of tumor TNM stages
3. Availability of tissues for microscopic analysis (glass slides or paraffin blocks)
4. Availability of peripheral blood cell analysis records 1 day before surgery and 3, 6 and 12 weeks after surgery;

The exclusion criteria were the following:
1. Other concomitant primary tumors
2. A history of chemotherapy or radiotherapy before surgery
3. A history of diseases interfering with white blood cell counts, including respiratory system failure, rheumatologic diseases, and active infectious diseases in recent months
4. Tumor with extensive ulceration or necrosis.

Demographic and clinical data of the patients were obtained from the hospital documents, including age, gender, tumor size, number of involved lymph nodes and tumor location.

One 3-μm section of surgically resected specimens of each tumor was stained routinely with haematoxylin and eosin. Grade of the tumor was determined [21]. In the next step, tumor-associated eosinophil counts were determined under a light microscope in ten random fields under ×40 magnification by two pathologists. All the specimens were examined through the entire depth. TATE was considered low (<50), moderate (50-120) and heavy (>120) in at least 10 HPFs [23].

Peripheral blood eosinophil count was obtained from CBC of patient files. For CBC tests, CBC counter CIS Mix (KX2 model, Japan) had been previously used. These data were obtained 1 day before surgery and 3, 6 and 12 weeks after surgery. CBC results were recorded after approval by an oncologist. As a result of a standard cut-off point, the researcher initially determined a cut-off point based on values obtained from 43 samplesand calculated to be 3%.

Data were analyzed by descriptive statistical methods (mean ± standard deviation), N (%) and repeated measures ANOVA for changes in the peripheral blood eosinophils, Pearson's correlation coefficient for correlation between peripheral blood TATE and tumor size and chi-square test for relation between peripheral blood TATE and tumor grade and lymph node involvement. Statistical significance was defined at p<0.05.

Results

In this study, 43 patients with oral SCC were studied; 23 patients (53%) were male and 20 (47%) were female. Average patient age was 59.89±11.12

The most common site of lesion was labial mucosa (12 cases), followed by buccal mucosa (10 cases), tongue (9 cases), floor of the mouth (8 cases), gingiva (3 cases) and palate (1 case). Twenty patients (47%) had lymph node involvement. The mean

tumor size was 7.51±0.96. Thirteen cases (30.23%) were grade 1, 19 (44.19%) were grade 2 and 11 (25.58%) were grade 3.

Patient peripheral blood eosinophil count average one day before surgery was 2.41±1.21, which changed to 2.78±1.39 twelve weeks after surgery (Table 1). During the first 3 weeks, changes were not statistically significant, but after 6 weeks (p=0.019) and 12 weeks (p=0.021), they were significant.

One day before surgery, 23 cases of peripheral blood eosinophil counts were less than 3% and others were more than 3%. Patient peripheral blood eosinophil count average one day before surgery was 2.41±1.21 and statistical analysis showed that there was a significant relationship between blood eosinophil counts and lymph node involvement (p=0.023). There was no relationship between peripheral blood eosinophil counts and tumor grade (p=0.12) (Table 2). In addition, statistical analysis showed that there was no correlation between blood eosinophil counts and tumor size (p=0.46).

Table 1. Peripheral blood eosinophil counts before, 3, 6 and 12 weeks after surgery in studied population

	Mean±SD	Range
Before surgery	2.41±1.21	0.70-5.00
3 weeks after resection	2.50±1.08	1.00-4.20
6 weeks after resection	2.89±1.19	1.00-5.00
12 weeks after resection	2.78±1.39	1.00-5.00

Table 2. Peripheral blood eosinophil counts according to tumor's grade and lymph node involvement

|	Blood eosinophil counts, n (%)		P_v
	3>	3<	
Grade			
I	8(61.54)	5(38.46)	
II	12(63.16)	7(36.84)	0.12
III	3(27.27)	8(72.73)	
Lymph node involvement			
Negative	16(73.91)	7(26.09)	0.023
Positive	7(40)	13(60)	

Grade II and III columns were merged for X_1^2 test

Table 3. Distribution of TATE according to tumor's grade and lymph node involvement

	Tissue Eosinophilia, n (%)			P_v*
	Low	Moderate	Heavy	
Grade				
I	4 (30.77)	3 (23.08)	6 (46.15)	
II	6 (31.58)	5 (26.32)	8 (42.10)	0.03
III	6 (54.55	3 (27.27)	2 (18.18)	
Lymph node involvement				
Negative	6 (26.08)	6 (26.08)	11(47.084)	0.10
Positive	10 (50)	5(25)	5(25)	

*Some cells were merged for X_1^2 test

TATE was low (<50) in 16 cases and was moderate (50– 120) and heavy (>120) in 11 and 16 cases, respectively (Figure1). Statistical analysis showed that there was a significant relationship between TATE and tumor grading (p=0.03). There was no relationship between TATE and lymph node involvement (p=0.10) (Table 3). Statistical analysis showed that there was no correlation between TATE and tumor size (p=0.83).
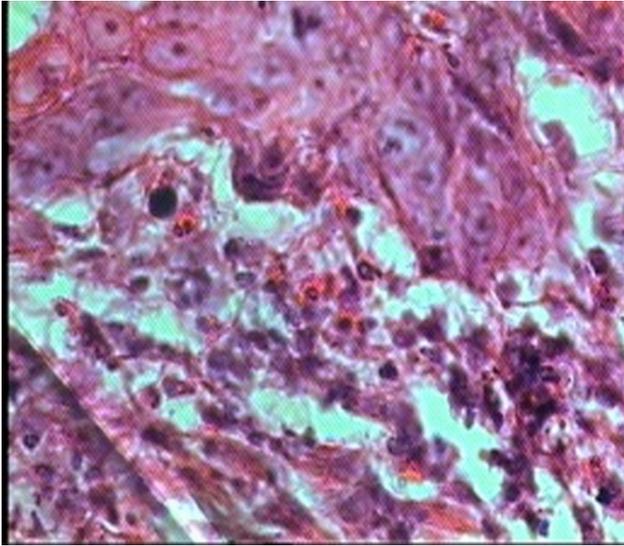
Figure 1. Oral squamous cell carcinoma showing rather numerous eosinophils around a nest of neoplastic oral epithelial cells. (H&E stained, original magnification ✗100)

Discussion

It is well established that eosinophilia, both in tissues and in the circulation, is the characteristic of many clinical conditions. Although the exact role of eosinophilia in cancers is not clear, it appears to occur as a result of four disease processes: (1) The tumor and/or its cell lines cause differentiation and proliferation of eosinophils in response to cytokines, which may be overproduced in malignant conditions. Cytokines have also been shown to prolong their lifespan. (2) Migration into the blood and tissues occurs and is directed to specific location by (3) chemoattraction. This is followed by (4) their activation and destruction [24].

The first case of malignant tumor associated with marked blood eosinophilia was described by Reinback [25], who reported a case of carcinoma of the neck associated with eosinophilia in 1893. Since then eosinophilia has been observed and described in many cases of carcinomas in various organs [24].

In the present study, for the first time the relationship between blood eosinophil counts and tumor size, lymph node involvement and grade was analyzed. There was a relationship between blood eosinophil counts and lymph node involvement; however, no significant association was found between blood eosinophil counts and the tumor size and grade.

Eosinophil counts in tissue samples revealed positive correlation with tumor grade without positive relation with tumor size and lymph node involvement. These findings are consistent with some studies and in contrast to some others.

The different results might be attributed to variations in the degree of activation of eosinophils present in different tumors, different methods of eosinophils staining, different sample sizes in different studies and different methods of eosinophil count determination.

Lowe et al [13] studied the relationship between TATE and clinical outcome in the head and neck tumors and reported that the patients with positive TATE had a good outcome. Consistent with Lowe et al [13], Thompson et al [26] and Deron et al [27] emphasized the presence of TATE as a favorable prognostic indicator in patients with head and neck tumors. Dorta et al [6] suggested anti-tumor role of eosinophils in OSCC.

However, in two recent studies by Tadbir et al [18] and Olivera [20] it was demonstrated that there wasn't any correlation between TATE and prognostic parameters and survival rate of OSCC patients. These findings suggest that intense TATE seems to reflect the stromal invasion of OSCC only in advanced clinical stages.

In contrast to these two studies, the majority of recent studies considered TATE as a histopathologic marker associated with tumor invasion and a clinical predictor for aggressive tumor biology [16,17].

Ercan et al [19] reported that there wasn't any correlation between TATE and prognostic parameters in laryngeal squamous cell cancer. They found that there is a high correlation between TATE and age and also reported that incidence of TATE is very low in over 60 year olds, which might suggest that age influences tissue inflammatory response to tumor.

Sassler et al [28] described an increased prevalence of TATE in tumors that showed marked lymphoplasmacytic infiltration. Contrary to the findings of earlier preliminary reports, they found no correlation between the presence of TATE and response to induction chemotherapy, overall survival rate and disease-free survival rate.

The results of the current study demonstrate that there is a relationship between TATE and tumor grade and between tumor-associated blood eosinophil counts and lymph node involvement. In addition, peripheral blood analysis has shown that blood eosinophilia counts before and 6 and 12 weeks after surgery were significantly different.

Based on a study by Lorena et al [7] hematoxylin/eosin was used for eosinophil staining in the present study but other staining methods can be used for eosinophil counts.

It was not possible in the present study to automatically quantify eosinophils with an image computer analyzer, which is considered a limitation of this study and it appears that use of a computer analyzer results in exact determination of eosinophil counts.

The results of the present study show that great attention should be paid to the role of eosinophils in tumor prognosis prediction but more prospective studies with long follow-ups are necessary to clarify the prognostic value of TATE and peripheral blood eosinophil counts in OSCC.

Conflict of interests: The authors declare no conflict of interest.

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