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Comparative Evaluation of Tissue Eosinophils Frequency in Oral Squamous Cell Carcinoma with and without Cervical Lymph Node Involvement

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ABSTRACT

Background and Objective: Oral squamous cell carcinoma (OSCC) is the most common oral cancer. The prognosis of OSCC depends on many factors. The stage of the tumor and the condition of the lymph nodes are two important factors in determining the treatment methods and prognosis. Since the identification of clinicopathological indicators, especially the cervical lymph node involvement, can increase the accuracy in estimating the prognosis and adopting the appropriate treatment method, the aim of this study is to compare the frequency of tissue eosinophils in OSCC with and without cervical lymph node involvement (CLNI).

Methods: In this cross-sectional study, 17 OSCC samples with CLNI (group 1) and 17 samples without CLNI (group 2) from the samples registered in the Pathology Department of Ayatollah Kashani Hospital, which were treated by neck dissection surgery, were selected and clinicopathological data were extracted. Then, in order to determine the number of tissue eosinophils, slides prepared with Congo red staining were blindly examined by two oral pathologists with an optical microscope.

Findings: 88.2% of the group with cervical lymph node involvement had lymphovascular involvement, while 88.2% of the group without lymph node involvement did not have lymphovascular involvement, and this significant difference was reported ($p<0.001$). Also, the mean number of tissue eosinophils in group 2 (19.95±5.67) was higher than group 1 (7.36±3.39), indicating a significant relationship with CLNI ($p=0.039$), but no significant relationship was found with any clinicopathological parameters.

Conclusion: According to the results of this study, the number of tissue eosinophils is an important prognostic factor in OSCC, and it can be used to better evaluate patients and provide more appropriate treatment.

Keywords: *Eosinophil, Squamous Cell Carcinoma, Mouth, Lymph Nodes*.

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Introduction

Cancer is one of the five major causes of death in all countries. 5% of all cancers occur in the head and neck, and almost half of these cases are in the oral cavity. Oral cancer is the eighth most common cancer in men and the fifteenth most common cancer in women (1). The variety of clinical manifestations may depend on the risk factors and anatomical conditions. We can observe different clinicopathological features in patients with different habits and methods of using tobacco (as the most important risk factor for oral cancer). Most patients are over 50 years of age (2). If oral malignancies are not diagnosed in the early stages, these lesions spread and cause irreparable deformities and dysfunction of the organ and ultimately lead to the death of the patient (3).

Squamous cell carcinoma (SCC) is the most common head and neck malignancy. In the past 30 years, the incidence of oral SCC (OSCC) is increasing especially in younger people despite the progress in diagnostic techniques and treatment methods (4-6). Many studies showed the relation between the location, extent of the tumor, stage of disease, histopathological grade and lymphovascular and nural invasion with the risk of metastasis to the cervical lymph nodes and the survival rate (2, 3). One of the investigated factors related to the possibility of metastasis to CLN and affecting the prognosis of OSCC patients are inflammatory cells. Inflammatory cells are mostly mononuclear cells and to a lesser extent neutrophils and eosinophils in tumoral tissue. Eosinophils originate from the bone marrow and include 1-3% of peripheral blood leukocytes (7). The presence of special spherical and oval shaped granules containing basic protein, eosinophil derived neurotoxin, eosinophil peroxidase and eosinophil cationic protein are the characteristics of this cell. Eosinophils have the ability to produce and secrete inflammatory mediators such as CSF-GM, interleukin 3 and 5, TNF-α, TGF-β and TGF-α, and some of these factors can cause the lysis of tumoral cells (8). An increase in the number of eosinophils has been seen in various head and neck diseases such as allergic gingivitis, lichen planus and cancers. Eosinophils also play a role in the biological behavior of carcinomas and produce substances to induce tumor progression or regulation. This cell plays a role in tumor lethality by releasing cytotoxic proteins directly and increasing the permeability of tumoral cells for the penetration of tumor-killing cytokines (9).

TATE (Tumor Associated Tissue Eosinophilia) means the infiltration of inflammatory cells with the majority of eosinophils around or inside the tumor and this inflammatory infiltration has occurred in many areas such as the pharynx, larynx, lungs, stomach, and oral cavity (10). Many studies have investigated the status of TATE and its relationship with the prognosis and survival rate of patients, but contradictory findings have been reported regarding the relationship between eosinophil infiltration and clinicopathological factors of head and neck SCC (11, 12). Most studies showed that TATE is a favorable prognostic marker in some malignant tumors and immunotherapy with the help of antitumor reaction of eosinophils can be used in cancer treatment. Considering the above explanations regarding the lack of agreement between research results and the lack of comparison of the status of this index in patients with and without metastasis, we decided to investigate the presence of eosinophils in oral SCC. Therefore, the aim of this study is to compare the frequency of tissue eosinophilia in oral squamous cell carcinoma with and without cervical lymph node involvement.

Methods

Our study protocol was approved by the Research and Ethics Committee of Isfahan University of Medical Sciences with ethics code: IR.MUI.RESEARCH.REC.1400.556. In this cross-sectional study, 34 samples were selected from the archives of the Pathology Department of Ayatollah Kashani Hospital with

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a definite diagnosis of OSCC, which were prepared by neck dissection treatment, and clinical data including age, gender, location and the size of the lesion was extracted from the files. 17 samples of OSCC with CLNI (N1) (Group 1) and 17 samples without CLNI (N0) (Group 2) were identified. Samples with incisional biopsy, lacking the necessary clinical information and lacking the proper quality of the block were excluded from the study. Then, the slides prepared by hematoxylin-eosin staining (H & E) from the oral lesions were examined by two oral pathologists with a light microscope for evaluation of tissue quality for Congo red staining and the histopathological grade of the tumor was determined based on the Bryne classification (13).

For Congo red staining, a 3-4 µ section was prepared and differentiated in 1% ethanol. The samples were washed with running water for 1 min and placed in 1% Congo red (Merck, Germany) aqueous solution for 20-30 min and they were placed in saturated lithium carbonate for 15 min and in 80% ethanol. Dehydration, clearing, and pasting were performed. All of the samples that were stained by Congo red staining techniques were evaluated simultaneously and blindly by two oral pathologists using an optical microscope (Olympus BX41TF, Tokyo, Japan) with 400× magnification. Ten non-overlapping fields were selected and the tissue eosinophils in the fields were counted. All data was entered into SPSS24 software and statistically analyzed by Chi-square, Kruskal-Wallis, Fisher exact and Mann-Whitney statistical tests. $P<0.05$ was considered as a significant difference.

Results

The mean age of all patients in this study was 59.64±17.02. According to Mann-Whitney test, there was no significant difference between two groups based on mean age of patients. In both study groups, the number of male patients (52.9%) was more than female patients and according to the chi-squared test, no significant difference was observed between OSCCs with and without CLNI. Tumor location, tumor size, depth of invasion (DOI), lymphovascular invasion (LVI), perineural invasion (PNI), stage of disease and histopathological grade were compared between two groups with and without CLNI. A significant difference was observed between the two study groups only in PNI and disease stage ($p<0.001$) (Table 1).

Table 1. Clinicopathological parameters in studied groups

Groups / Parameters	OSCC with CLNI Number(%)	OSCC without CLNI Number(%)	p-value (test)
Age (Mean±SD)	59.58±19.04	59.7±15.34	0.871 (t-test)
Gender			
Male	9(52.9)	9(52.9)	1.0
Female	8(47.1)	8(47.1)	(Chi-square)
Site			
Tongue	11(64.7)	13(76.5)	
Alveolar mucosa	5(29.4)	2(11.8)	0.189
Buccal mucosa	0(0)	2(11.8)	(Fisher's exact test)
Floor of mouth	1(5.9)	0(0)	
Size (Mean±SD)	2.27±1.25	2.25±1.38	0.521 (t-test)
DOI[1]			
<5 mm	6(35.3)	6(35.3)	0.315 (Mann-Whitney)
5-10 mm	3(17.6)	8(47.1)	
>10 mm	8(47.1)	3(17.6)	

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LVI[2]			
Positive	15(88.2)	2(11.8)	<0.001
Negative	2(11.8)	15(88.2)	(Chi-Square)
PNI[3]			
Positive	13(76.58)	11(64.7)	0.452
Negative	4(23.5)	6(35.3)	(Chi-Square)
Grade			
Well differentiated	11(64.7)	10(58.8)	0.98
Moderate differentiated	6(35.3)	5(29.4)	(Mann-Whitney)
Poorly differentiated	0(0)	2(11.8)	
Stage			
Stage I	0(0)	7(41.2)	
Stage II	0(0)	8(47.1)	<0.001
Stage III	14(82.4)	2(11.8)	(Mann-Whitney)
Stage IVA	3(17.6)	0(0)	

1: Depth of invasion, 2: lymphovascular invasion, 3: Perineural invasion

According to Mann-Whitney test, there is a statistically significant difference in the frequency of tissue eosinophils between the two groups (p=0.039). The number of eosinophils in group 2 was more than group 1 (Table 2). Also, the mean number of tissue eosinophils in patients under 40 years old was higher than in patients over 40 years old, although this difference was not statistically significant. On the other hand, the mean number of tissue eosinophils was higher in women than in men, although this difference was not statistically significant. Based on Spearman's correlation analysis, the correlation between tumor size and tissue eosinophils frequency did not show a significant relationship (r=-0.006, p=0.975). In the present study, the mean number of tissue eosinophils based on the location (p=0.27), DOI (p=0.391), LVI (p=0.2), PNI (p=0.26), histopathological grade (p=0.095) and disease stage (p=0.16) did not have a statistically significant difference (Figure 1).

Table 2. Eosinophils frequency based on clinicopathological parameters

Parameters	Eosinophils (Mean±SD)	p-value (test)
Groups		
Group1 (OSCC with CLNI)	7.36±3.39	0.039
Group 2 (OSCC without CLNI)	19.95±5.67	(Mann-Whitney)
Age		
<40 Years old	21.1±14.99	0.129
≥40 Years old	12.37±20.72	(Mann-Whitney)
Gender		
Male	10.28±14.79	0.615
Female	16.66±23.78	(Mann-Whitney)
Location		
Tongue	14.66±21.88	
Alveolar mucosa	2.5±2.9	0.27
Buccal mucosa	32.5±17.68	(Kruskal-Wallis)
Floor of mouth	30±0	

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DOI[1]		
<5 mm	12.75±20	0.391 (Kruskal-Wallis)
5-10 mm	18.37±25.41	
>10 mm	9.94±14.08	
LVI[2]		
Positive	9.1±14.44	0.2 (Mann-Whitney)
Negative	18.19±23.99	
PNI[3]		
Positive	22.52±28.23	0.26 (Mann-Whitney)
Negative	9.97±14.68	
Grade		
Well differentiated	15.67±22.45	0.95 (Kruskal-Wallis)
Moderate differentiated	5.9±10.3	
Poorly differentiated	35±21.21	
Stage		
Stage I	24.51±30.51	0.16 (Kruskal-Wallis)
Stage II	18.9±17.93	
Stage III	6.4±12.37	
Stage IVA	7.06±11.2	

1: Depth of invasion, 2: lymphovascular invasion, 3: Perinural invasion

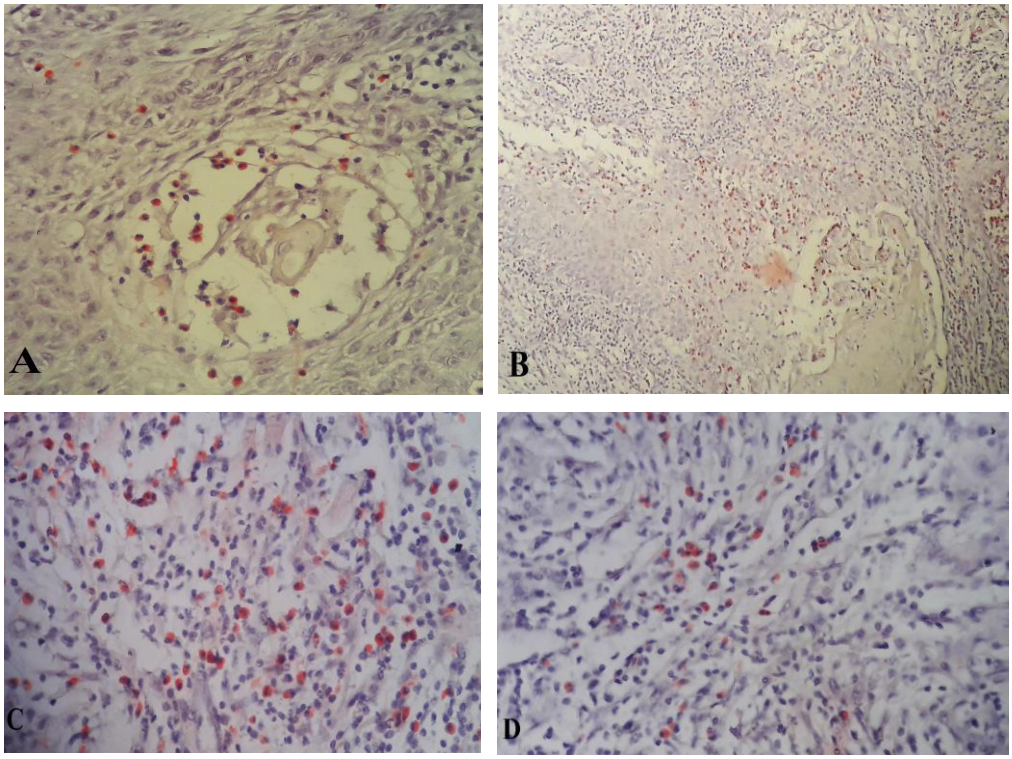

Figure 1. A: Tissue eosinophils in well differentiated OSCC (Congo red staining, ×400), B: Tissue eosinophils in moderately differentiated OSCC (×100), C: High frequency of eosinophils (×400), D: low frequency of eosinophils (×400)

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Discussion

Involvement of cervical lymph nodes is one of the important influencing factors in treatment results and prognosis of oral cancer (14). In the present study, a significant relationship was found between the stage of the disease and CLNI, which is consistent with the studies of Li et al. (15) and Gadbail et al. (16), but it is in conflict with the results of Wang et al. (17). Moreover, CLNI had a significant relationship with lymphovascular involvement, which is contrary to the results of the studies of Suresh et al. (18), Li et al. (15) and Chen et al. (19), but in line with the study of Wu et al. (20), Adel et al. (21) and Sahoo et al. (22). Lymphovascular and perineural invasion are considered as important pathological factors. Blood vessels invasion by tumoral cells is one of the first stages of metastasis (15). In this study, no significant relationship was found between CLNI and other clinicopathological parameters.

In the present study, eosinophils frequency in group 2 was significantly higher than group 1, which is consistent with the study of Jain et al. (23). However, it is in conflict with the results of the study by Alaeddini et al. (24) and Yellapurkar et al. (25) who did not report a significant difference. The results observed in the recent studies were different and this difference was due to the difference in the groups evaluated in the studies. The results show a definite relationship between the tumor microenvironment, its progression and the involvement of the cervical lymph nodes. A significant relationship between the density of immunological cells and the reduction of cervical lymph node involvement has been stated in studies. Accordingly, the increase in the number of immunological cells including tissue eosinophils (TATE) is a favorable prognostic indicator in OSCC (24, 25).

In the present study, the mean number of tissue eosinophils in younger patients was reported to be higher than older patients, although the difference was not significant, which was in line with the study of Rahrotaban et al. (26), while it was significant based on the age of the patients in the study of Debta et al. (27). In line with the present study, in the studies of Rehrotaban et al. (26), Debta et al. (27), Naderi et al. (28), no significant relationship was observed between the mean number of eosinophils and gender. The difference of this cell based on the tumor location was not significant in this study, which is in accordance with the studies of Rehrutaban et al. (26) and Debta et al. (27).

In the present study, the mean number of tissue eosinophils in samples without LVI and with PNI was high, while in the study of Amouian et al, it was higher in patients with perivascular invasion (29). In the present study, the highest number of eosinophils was found in poorly differentiated OSCCs and the lowest in moderately differentiated OSCCs, although no significant difference was observed between histopathological grade and eosinophils. In the study of Rehrutaban et al., the frequency of tissue eosinophilia in the group of patients with poorly differentiated head and neck SCC was lower than the other two groups (26). In the study of Naderi et al., the mean number of eosinophils in well-differentiated OSCC was higher than in moderately differentiated samples, but a significant relationship was not found (28). In the studies of Siddiqui et al. (30) and Deepthi et al. (31), eosinophils frequency in poorly differentiated tumors was significantly higher than in other groups However, in the study of Debta et al., from well tumor differentiation to poor differentiation, the number of eosinophils decreased (27). In the studies of Yellapurkar et al. (25) and Joshi et al. (32) the eosinophils level was higher in well-differentiated samples, but there was no significant relationship. Differences in the investigated samples and the method of detection of tissue eosinophils are the reasons for the different results in different studies.

In the present study, the highest mean number of eosinophils was observed in stage I and II. In the study by Choudhary et al., high tissue eosinophilia was associated with positive overall survival and negative disease-free survival in OSCC (33). In the study by Debta et al., the number of eosinophils decreased from

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stage I to stage III (27), all of which were consistent with the present study. In the study of Nishikawa et al., they showed that the increase in the tissue eosinophils after the administration of nivolumab in patients with head and neck SCC leads to a better prognosis and longer survival of patients (34).

Eosinophil infiltration in the tumoral tissue is carried out by production of several chemokines of the tumoral cells. Tissue expression of eotaxin secreted by eosinophils has been associated with increased eosinophil migration in Hodgkin lymphoma. In OSCC, eotaxin is mainly secreted by eosinophils, suggesting an autocrine and/or paracrine pathway to maintain tissue eosinophilia. In addition, damage-associated molecular pattern (DAMP) molecules released by necrotic tumor tissue stimulate eosinophil migration. HMGB1 (High-mobility group box 1) released from necrotic tumor tissues recruit eosinophils through a receptor for advanced glycation end products expressed on eosinophils and induces degranulation (27). Therefore, a high eosinophil count indicates an increase in tumor antigens due to tumor necrosis and collapse. An increase in tumor antigens can lead to a high therapeutic response to immunotherapy. Several studies have shown a correlation between increased eosinophils during treatment and longer survival of patients (32-34)

Tissue eosinophils frequency was higher in OSCC without CLNI. Eosinophils is important as a prognostic parameter in OSCC, and it can be used to better evaluate patients with this tumor and provide more appropriate treatment.

Conflict of interest: The authors have no conflict of interest.

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