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Tissue eosinophilia in oral and cutaneous squamous cell carcinoma and normal oral and cutaneous tissues

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Abstract

Introduction: Eosinophils produce proteases that stimulate invasion in cancers. Due to the more malignant potential of oral squamous cell carcinoma (OSCC) compared to cutaneous squamous cell carcinoma (CSCC), the aim of the present study was to compare tissue eosinophilia between OSCC and CSCC samples to understand whether tissue eosinophilia play a role in different biological behavior of OSCC and CSCC. Also, we assessed the relation between clinical stage and mean eosinophil counts.

Methods: This cross-sectional study included histopathological slides of 30 OSCC, 30 CSCC, 15 normal skin and 15 normal oral mucosa samples. The mean number of eosinophil per square millimeter (eos/mm^2) was calculated and the severity of tissue eosinophilia was accordingly categorized into absent/mild and sever. Clinical stages of OSCC and CSCC samples were extracted from archived files.

Results: There was significant relationship between mean eosinophil count and clinical stage in both OSCC and CSCC groups (P<0.0001).The differences of mean numbers of eos/mm^2 in OSCC and CSCC groups compared to normal groups were significant (P = 0.001 and P = 0.001, respectively), but when we compared OSCC and CSCC groups, the difference was not significant. OSCC and CSCC groups showed significant difference in severity of tissue eosinophilia compared to normal groups (P=0.005 and P =0.004, respectively), but in comparison of OSCC and CSCC groups, the difference was not significant.

Conclusion: Intense tissue eosinophilia is correlated with stromal invasion in OSCC and CSCC samples as seen in advanced clinical stage. Different biological behaviors of OSCC and CSCC do not depend on eosinophils and other factors can be involved.
developed in the left eye. Two patients had no family history suspicious for keratoconus.

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Introduction

Oral squamous cell carcinoma (OSCC) is the most common oral malignancy and cutaneous squamous cell carcinoma (CSCC) is the second most common skin cancer (1-2). In solid tumors, such as oral squamous cell carcinoma, a combination of the effects of cancer cells and stromal cells (i.e. fibroblasts, endothelial cells and inflammatory cells) has been considered as being involved that in harmony with each other act towards tumor progression, angiogenesis, local invasion and metastasis (3).

Eosinophils are a rare subset of inflammatory cells (granulocytes) that are involved in the pathogenesis of many important diseases including allergies and parasitic infections (4). It has been hypothesized that eosinophils have direct and indirect influences on tumor progression. Eosinophils may stimulate tumor angiogenesis. These cells also contain matrix metalloproteinases (MMPs) such as MMP-9 and through them, can also regulate the formation of extracellular matrix (5).

On the other hand, malignant and metastatic potential of OSCC and CSCC are different (6). OSCC and CSCC differ in terms of their prognosis. In OSCC, 5- year survival rate varies between 35% and 45% and the risk of metastasis varies between 40% and 50% (7, 8). In head and neck CSCC, the risk of metastasis is 11.7% and 5-year survival is 54% (9, 10). The aim of the present study was to compare tissue eosinophiliabetween OSCC and CSCC samples, to understand whether tissue eosinophiliaplay a role in different biological behavior of these two malignancies.

Methods

This cross-sectional analytical-descriptive study included 90 samples (30 OSCC, 30 CSCC, 15 normal oral mucosa and15 normal skin samples). We studied tissue eosinophils in these samples (Figures 1 and 2). Hematoxylin-eosin stained histopathological slides were examined under an optical microscope (Olympus BX41,Japan) with a magnification of 400X and the number of eosinophils in 15 successive fields was counted at the invasive front of oral and cutaneous squamous cell carcinoma and the mean number of eos/mm^{2}wascalculated; Also, the number of eosinophils in 15 successive fields in sub-epithelial connective tissue in normal oral mucosa (NO) and normal skin (NS) samples was counted and the mean number of eos/mm^2 was calculated.

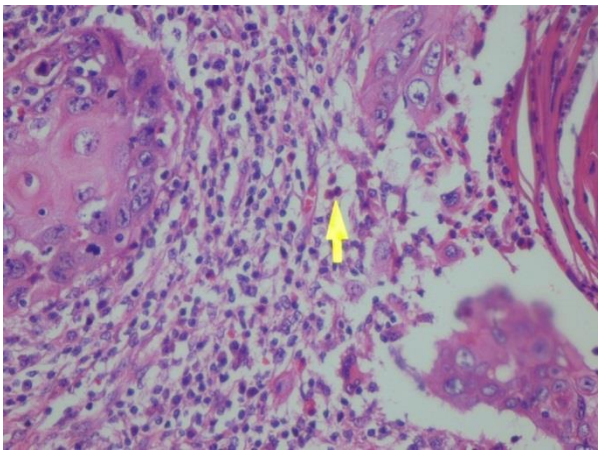

Figure 1. Tissue eosinophils in invasive front of OSCC (400X magnification)

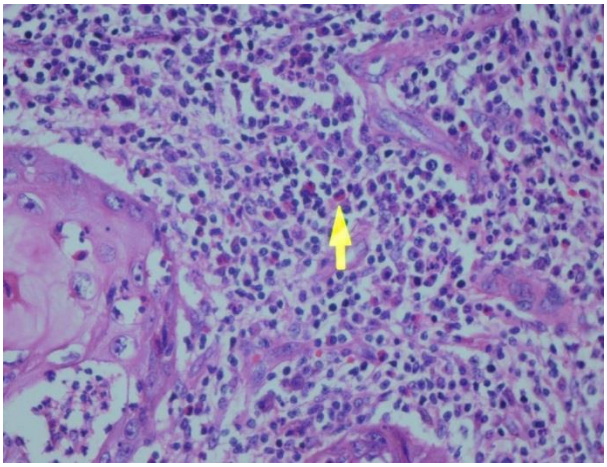

Figure 2. Tissue eosinophils in invasive front of CSCC (400X magnification)

The severity of tissue eosinophilia categorized into two fallowing groups: absent/mild (0-67 eos/mm^2) and sever (≥68 eos/mm^2) (11).

Clinical stages of OSCC and CSCC samples were extracted from archived files and divided into early stage (stage I and II) and advanced stage (stage III and IV) (12).

The obtained data were entered in SPSS software and analyzed by statistical tests (Chi-Square, Mann-Whitney and t-test). Significant level was considered as $p<0.05$.

It should be mentioned that sample size calculation was carried out according to similar researches in this area (5, 12-20).

Ethical Approvals

The study has been independently reviewed and approved by ethical board of BabolUniversity of Medical Sciences (Code: 2851).

Results

The patients' demographic data have been reported in table 1. In CSCC and NS groups, the majority of samples belonged to male patients, but in OSCC and NO groups, most cases were female and younger.

The mean number of eos/mm^{2}inOSCC, CSCC, NO and NS groups have been summarized in table 2.

According to t-test results, there was significant relationship between mean eosinophil count and clinical stage in OSCC group ($P<0.0001$), so that OSCC samplesbelonged to advanced clinical stages showed significantly higher mean eosinophil count (table 3).

Also, Mann-Whitney test showed significant relationship between mean eosinophil count and clinical stage in CSCC group ($P<0.0001$), so that CSCC samples belonged to advanced clinical stages showed significantly higher mean eosinophil count (table 3).

Table 1. Age, sex and site distribution of patients

Group	site	n	Sex		age	
			Male	Female	<50	≥50
CSCC***	face	6	26	4	6	24
	hand	1				
	leg	2				
	ear	1				
	lip	3				
	scalp	16				
	trunk	1				
NS****			11	4	9	6
OSCC*****	tongue	16	12	18	10	20
	Anterior site*	3				
	Posterior site**	11				
NO******			6	9	14	1

*Anterior site: tissue located anterior to the line connected two canines for each jaw

**Posterior site: tissue located posterior to the line connected two canines for each jaw

***CSCC: Cutaneous squamous cell carcinoma

****NS: Normal skin

*****OSCC: Oral squamous cell carcinoma

******NO: Normal Oral mucosa

Table 2. Mean number of eos/mm2 in studied groups (Confidence Interval= 95%) (using Mann-Whitney test)

Group	N	Mean	S.D.	Z-value	p-value
OSCC*	30	114.6917	152.22188	-3.516	0.000
Normal oral tissue	15	15.7193	18.01881		
CSCC**	30	99.1913	104.39897	-4.062	0.000
Normal skin tissue	15	11.7747	35.14251		

*OSCC: Oral squamous cell carcinoma

**CSCC: Cutaneous squamous cell carcinoma

Table 3. Mean eos/mm2 in diiferent stages of studied groups (Confidence Interval= 95%) (using Mann-Whitney test and T-test)

Group		N	Mean	S.D.	Mean Rank	p-value
OSCC*	early	15	31.2333	17.80135	-	<0.0001
	advanced	15	211	50.38518	-	
CSCC**	early	15	27.4	20.30060	8	<0.0001
	advanced	15	182	61.79941	22	

*OSCC: Oral squamous cell carcinoma (using T-test)

**CSCC: Cutaneous squamous cell carcinoma (using Mann-Whitney test)

The results of Mann-Whitney test showed significant difference in the number of eos/mm² between OSCC and NO groups (p= 0.001) and also between CSCC and NS groups (p=0.001), but the difference between CSCC and OSCC groups was not statistically significant (p= 0.745). In terms of the severity of tissue eosinophilia (absent/mild and severe), Chi-Square test showed no significant difference between OSCC and CSCC groups (p=0.217), but

the differences between OSCC and NO groups (p=0.005) and between CSCC and NS groups (P =0.004) were significant (table 4).

Table 4. Classification of severity of tissue eosinophilia in studied groups(using Chi-Square test)

Group	N	Severity of tissue eosinophilia		P-value
		Mild/ absent 0-67 eos/mm^2	Intense ≥68 eos/mm^2	
Normal oral tissue	15	15 (100%)	0 (0%)	0.005
OSCC*	30	19 (63.3%)	11 (36.7%)	
CSCC**	30	15 (50%)	15 (50%)	0.004
Normal skin tissue	15	14 (93.3%)	1 (6.7%)	

*OSCC: Oral squamous cell carcinoma

**CSCC: Cutaneous squamous cell carcinoma

Discussion

Due to different malignant potential of OSCC and CSCC, in this study, we attempt to compare the severity of tissue eosinophilia and mean number of eos/mm^2 between these two malignancies to understand whether tissue eosinophilia plays a role in different biological behavior of OSCC and CSCC or not.

In our study, there were significant relationship between mean eosinophil count and clinical stages in both OSCC and CSCC groups (P<0.0001), so that OSCC and CSCC samples belonged to advanced clinical stages showed significantly higher mean eosinophil count. This means that intense tissue eosinophilia is correlated with stromal invasion in OSCC and CSCC.

In this research, we found significant differences in the mean number of eos/mm^2 and severity of tissue eosinophilia between OSCC and NO groups and between CSCC and NS groups. Significant difference in the average number of eos/mm^2 and severity of tissue eosinophilia between OSCC and NO groups suggest increased presence of these inflammatory cells in OSCC and probably their role in tissue invasion process and progression of OSCC. This finding is consistent with the results of almost all studies in this area (5, 12-19). Significant difference of CSCC and NS groups' in the average number of eos/mm^2 and severity of tissue eosinophilia suggest increased presence of these inflammatory cells in CSCC and probably their role in tissue invasion process and progression of CSCC. This finding is consistent with the results of Lowe et al study (20).

No significant difference was found between OSCC and CSCC groups in the average number of eos/mm^2 and severity of tissue eosinophilia. Although the malignant and metastatic potential of OSCC is more than CSCC, but according to our findings more invasive potential and poor prognosis of OSCC compared to CSCC are due to some factors except tumor associated tissue eosinophilia; therefore the

role of eosinophils in different biological behavior of OSCC and CSCC is doubtful. Perhaps, more aggressive behavior of OSCC compared to CSCC is related to factors such as more vascularity of oral cavity compared to skin and subsequent easier access to lymphatic and blood vessels for earlier metastasis, late diagnosis of OSCC compared to CSCC due to less visibility, lack of safe margins and less capability to resect the entire tumor in oral cavity compared to skin due to more vicinity of oral cavity to vital organs and less accessibility in mouth and finally related to other molecules, markers and cells. Because of the novelty of current study in such a field (comparison of the average number of eosinophils and severity of tissue eosinophilia between OSCC and CSCC groups) and lack of previous similar studies, it is not possible to compare this study with other studies from this standpoint.

In our findings, the average number of eos/mm^2 and severity of tissue eosinophilia in tongue OSCCs were significantly higher than OSCCs in other oral sites. With respect to more aggressive behavior and poorer prognosis of tongue OSCC compared to OSCC of other oral sites (21), perhaps more malignant potential of tongue OSCC may be related to higher eosinophilic infiltration in stroma of tongue OSCC than OSCC of other oral sites. Also, it can be concluded that probably more eosinophilic infiltration in stroma of OSCC is predictor of poorer prognosis of OSCC.

Conclusion

Intense tissue eosinophilia is correlated with stromal invasion in OSCC and CSCC as seen in advanced clinical stage. It can be concluded that although the presence of eosinophilic infiltration probably helps the progression and invasion of OSCC and CSCC, but it does not have much important role in different biological behavior of OSCC and CSCC.

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