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Medical Journal of the Islamic Republic of Iran (MJIRI)
Iran University of Medical Sciences

A study on the relationship between clinical features with Ki67 expression and eosinophil cells infiltration in oral squamous cell carcinoma

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Received: 13 January 2014 Accepted: 16 March 2014 Published: 20 October 2014

Abstract

Background: Cell proliferation is one of the most critical factors in metastasis and prognosis of the malignant tumors.Recent investigations show that the eosinophil granolosytes have an important role in developing of malignant tumors. The relation between cell proliferation and eosinophilic infiltration in oral squamous cell carcinoma (OSCS) with prognosis is unclear. The aim of this study was to investigate the relationship between the Ki67 expression and eosinophilic infiltration with the clinical features on OSCC.

Methods: This study was cross sectional in which 24 paraffined embeded block of OSCC selected from the Imam Khomeini hospital; cancer institute's archive. 4 micron sections were prepared and studied for Ki67 antigen immunohistochemically. The labeling index (LI: positive epithelial cells/1000 epithelial cells) of Ki67 positive cells were obtained. In each section eosinophilic cells were counted in 10 fields with 400 (HPF). The relations between the eosinophil cells and Ki67 positive cells counts with clinical features and histopathological differentiation were achived by the linear regression and spirman statistical tests.

Results: There were no any significant relationship between gender, histopathological differentiation and the number of eosinophils and Ki67 positive cells counts (p= 0.33 and p=0.73). A significant relationship between lymph node involvement and the number of eosinophils and the Ki67 positive cells counts was found (p=0.04).There was a positive relationship between the number of Ki67 positive cells and the number of eosinophil cells (p= 0.05).

Conclusion: A significant relationship between lymph node involvement with eosinophilc cells and Ki67 positive cells counts were exist.

Keywords: Eosinophil cells, Lymph node involvement, Oral squamus cell carcinoma, Ki67 antigen.

Cite this article as: Jalayer Naderi N, Tirgari F, KharaziFard M.J, Farahani Parsa F. A study on the relationship between clinical features with Ki67 expression and eosinophil cells infiltration in oral squamous cell carcinoma. *Med J Islam Repub Iran* 2014 (20 October). Vol. 28:115.

Introduction

Squamous Cell Carcinoma (SCC) is the most common malignant epithelial tumor of oral cavity. Uncontrolled proliferation, lymph node involvement and metastasis are potential outcome of oral SCC (1).

Cell proliferation is an important factor in tumor aggressiveness. It has been shown that cell proliferation has a strong positive relationship with the pathologic grade and prognostic factors in S CC (2, 3).

The Ki67 antigen defined as a reliable marker for demonstrating cells proliferation. Except G0 phase (cells in rest phase), it is expressed in all cycle cell phases (4).

Previous studies showed the relation between Ki-67 expression and tumor prognosis in different malignancies (5-7).

Eosinophils are one of the immune system members that involved in bactericidal

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and allegic activities. It has been demonstrated that tissue eosinophilia is associated with different epithelial originated malignancies (8-11).

In spite of these reports, the function of eosinophilic cells in tumor outcome is not clear. It seems that tumor -associated tissue eosinophilia (TATE) is a tumor – related reaction (12).

The aim of study was to investigate the relationship between the Ki67 expression and eosinophilic infiltration with clinical features in OSCC. Based on our search, this is the first study on investigating the cell proliferation and eosinophilic infiltration in OSCC.

Methods

The study was retrospective with based on archive sampling. By reviewing the medical records of patients with OSCC and examining the hematoxilin-eosin stained slides, 24 formalin-fixed, paraffin embedded samples of OSCC were selected and retrieved from the archive of dept. of Pathology, Cancer Institute, Imam Khomeini hospital, Tehran. Some samples were excluded because of previous radiotherapy or chemothrapy history, incomplete medical record information and inadequate tumoral material. Patients' age, gender, lymph node involvement and tumor size were registered for all samples.

The 3 µm sections were prepared for investigating the ki67 expression. The sections were treated as follows: deparaffinized in xylene, placing in 0.01 M Citrate/HCl Buffer (pH= 6.00), and then heated in microwave oven for 10 minutes, rinsing with phosphate buffered saline (PBS),incubation with 1µg/ml diluted primary antimouse monoclonal antibodies (Dako, Denmark Ki-67) for 1 hour and then with biotinylated antibody for 30 minutes, incubated with peroxidase for 30 minutes and develope in 3,3'diaminobenzidine hydrochloride (DAB). The final step was Mayer's staining, immersion in xylene and mounting. Between incubation periods, all samples were rinsed with PBS. The Phaeochromocytoma tumor was used as positive control.

The Ki-67 expression was assessed by obtaining the labeling index (LI: positive epithelial cells/1000 epithelial cells) for each section.

In each section eosinophil cells were counted in 10 fields.The count was compeleted by using hematoxilin-eosin stained slides (13).

The quantifications were blind and completed by light microscopy (Ziess, Japan) at × 400 magnifications. The relations between the eosinophil cells and Ki67 positive cells counts with clinical features and histopathological grade were achived. Linear regression, t-test and spirman tests were used for statistical analyzing. The SPSS 13.0 software (SPSS Inc., Chicago, USA) was employed, and $p< 0.05$ was considered as statistical significance level.

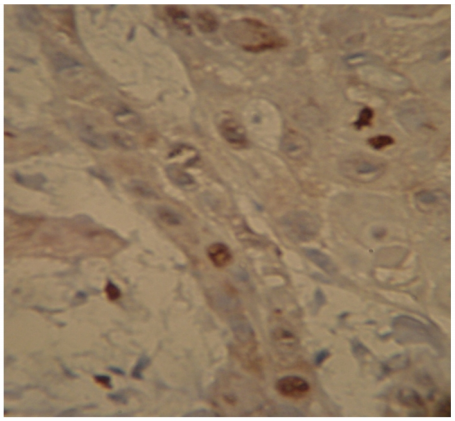

Fig. 1.Immunohistochemical staining of Ki-67 expression in epithelial cells of OSCC (×400)

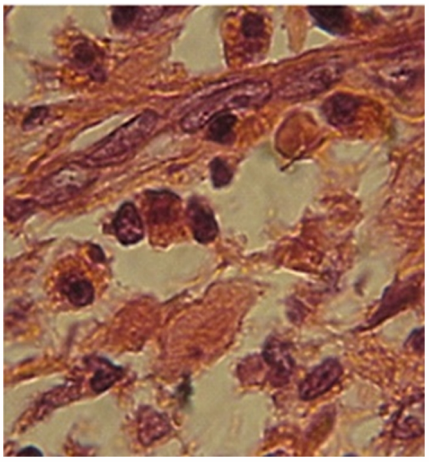

Fig. 2. Eosinophil cells within connective tissue (Hematoxilin-Eosin staining, ×400)

Tabe 1. Summary of clinical features, Ki67 LI and eosinophil cells count

	Gender	Age	Size	Lymph node involvement	Histopathologic grade	Ki67 LI	Eosinophil cells count
1	Female	44	1.5	Yes	Moderate	398	58
2	Female	70	2.5	Yes	Well	408	35
3	Female	41	3	No	Well	61	9
4	Female	68	4.5	No	Moderate	92	2
5	Male	25	2	Yes	Well	600	35
6	Male	82	2	Yes	Moderate	283	40
7	Male	69	1.3	Yes	Well	444	22
8	Male	71	3	Yes	Well	529	29
9	Male	85	5.5	No	Well	498	6
10	Female	71	6	Yes	Well	576	15
11	Female	81	6	Yes	Well	483	36
12	Male	52	4	No	Moderate	86	6
13	Female	85	2	Yes	Moderate	377	20
14	Female	31	2	No	Moderate	100	4
15	Male	39	5.5	No	Well	120	12
16	Male	70	5	Yes	Well	200	71
17	Female	50	3	No	Well	133	8
18	Male	28	3	Yes	Well	299	25
19	Female	38	2.5	No	Well	61	5
20	Male	64	2	Yes	Well	214	38
21	Male	60	3	No	Well	70	4
22	Female	58	2.5	No	Well	83	0
23	Female	70	4	Yes	Well	500	29
24	Male	48	2	No	Well	83	1

Results

The Ki-67 positive cells were experessed light to dark brown color of nuclei in basal and squamous layer of epithelium (Fig. 1).

Eosinophils were nucleated cells with intense granules in the cytoplasm (Fig. 2).

There were 12 (50%) males and 12 (50%) females with the mean age of 58.33 years. 21 (%87.5) of samples were from tongue and 3 (%12.5) from floor of the mouth.

18 (75%) were well-differentiated squamous cell carcinoma and 6 (25%) moderate squamous cell carcinoma. There were 13 (45.8%) lymph node involvement. 11 (54.2%) were not involved with lymph node metastasis. Tumor size, by considering the largest diameter, was 1.5 to 6 cm. Tabel 1 shows the summary of clinical features, Ki67 LI and eosinophil cells count. Table 2 shows the frequency (mean rank) of ki67 expression and eosinophil cells count in oral squamous cell carcinoma according to tumor differentiation, lymph node involvement and gender.

The correlation between lymph node involvement with Ki67 LI and eosinophil cells count was significant (p= 0.04).

The correlation between gender with Ki67 LI and eosinophil cells count was not significant (p= 0.33).

The correlation between tumor differentiation with Ki67 LI and eosinophil cells count was not significant (p= 0.37).

The correlation between tumor size with Ki67 LI was not significant (p= 1.04), but the correlation was significant in related to eosinophil cells count (p= 0.0003).

A positive correlation of Ki-67 expression and eosinophil cells count was obtained (p= 0.05).

The area under ROC curve was 0.937 and 1 for Ki67 LI and eosinophil cells count, respectively (Fig. 3). By comparison of the corresponding points in ROC curve, cut-of point= 166.5 with positive predictive value 92.9%, negative predictive value 100%, specifity of 91.9% and sensitivity of 100% were achieved for ki67 expression in relation to lymph node involvement. The cut-of point = 13.5 was achieved for eosinophil cells count in relation to lymph node involvement with specifity and sensitivity of 100%.

Disscusion

In this study, significant relationship be-

Table 2. The frequency (mean rank) of ki67 expression and eosinophil cells count in oral squamous cell carcinoma according to tumor differentiation, lymph node involvement and gender

	No.	Min.	Max.	Mean	Std. Deviation
SCC differentiation					
Ki67 Li	6	86	398	222.67	147.648
Eos.count	6	2	58	21.67	22.818
Ki67 Li	18	61	600	297.89	203.302
Eos.count	18	0	71	21.11	18.136
Lymph node involvement					
Ki67 Li	11	61	498	126.09	125.373
Eos.count	11	0	12	5.18	3.573
Ki67 Li	13	200	600	408.54	130.568
Eos.count	13	15	71	34.85	15.345
Gender					
Ki67 Li	12	61	576	272.67	200.086
Eos.count	12	0	58	18.42	17.763
Ki67 Li	12	70	600	285.50	189.672
Eos.count	12	1	71	24.08	20.300

tween lymph node involvement with eosinophilc cells and Ki67 positive cells counts was exist. Significant association between gender and tumor differentiation with Ki67 LI and eosinophil cells count were not found.

The reports in regard to tumour-associated tissue eosinophilia in oral cavity/upper digestive system is limited. In one study, Ishibashi et al. reported that tumor-associated eosinophils count had a significant correlation with vascular invasion, lymph node metastasis and recurrence in esophageal squamous cell carcinoma (11).

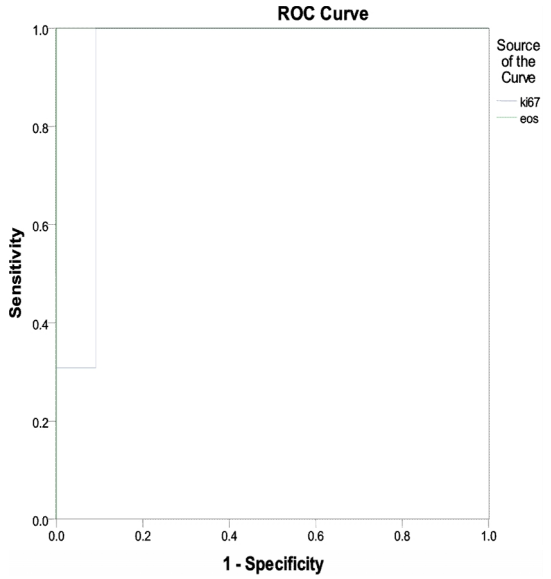

Fig. 3. The area under ROC curve were 0.937 and 1 for Ki67 Li and eosinophil cells count, respectively

The observation of Falconieri et al. on 13 cases of oral squamous cell carcinoma showed that the tumors with higher eosinophils are associated with stromal invasion and cervical lymph node involvement (14).

These findings are compatible with our results that showed a significant correlation between lymph node involvement with eosinophilc cells counts.

In addition to esophageal and oral squamous cell carcinoma, tumor-associated eosinophilic infiltration is also related to prognosis in gastric cancer, cervical cancer and laryngeal squamous cell carcinoma (15-17).

Some studies are focused on indicating the possible role of eosinophilc cells counts in metastasis and invasion as an accessory aid in determining the malignant tumor prognosis.

It has been shown that in squamous cell carcinoma of the aerodigestive tract, the eosinophilic counts higher than10 eosinophils/HPF had predictive value for invasion with 66% and94% sensitivity and specifici-

ty, respectively (13).

Said et al. reported that eosinophils count more than10/ HPF or>20/10 HPF with sensitivity of 82% and specificity of 93%, can be an indicator for invasion of laryngeal squamous neoplasia (10).

The results of this study show that the eosinophils count≥ 13.5 with 100% sensitivity and specificity was an indicator of lymph node metastasis.This finding in agreement with mentioned above, designate the importance of eosinophils count for predictating the presence of tumor invasion. This is an important finding because in small specimens the number of eosinophils is able to be a histopathologic aid for determining the presence of invasion.

In this study, there was not any correlation between age, gender and degree of differentiation to eosinophils count and ki67 antigen expression. This is compatible with Sassler etal. report on advanced laryngeal squamous carcinoma (17).

Contrary to these findings, Ercan et al. found a positive correlation with eosinophilic infiltration and age in laryngeal squamous cell cancer (18).

This different finding is mainly due to different number of cases and study plan.

Van Driel et al. belived that the presence of high amount of eosinophilic infiltration in patients with tumor-negative lymph nodes was an independent parameter and compatible with a poorer survival. This related to inappropriate immune response (16).

Obtaining more conclusive results on eosinophilic infiltration related to prognostic factors in different tissues needs further cases and multicenteric studies.

This study compatible with earlier studies that have been shown the ki67 antigen expression on oral squamous cell carcinoma (19-20).

The ki67 antigen expression was shown from epithelial dysplastic lesion to squamous cell carcinoma (20-22).

In spite of these findings, present data about the relation between ki67 antigen expression and clinical variations is insufficient.

In this study, there was no significant relationship between gender, histopathological differentiation with Ki67 expression on oral squamous cell carcinoma.

This is in agreement with Sun et al. that found Ki67 expression in squamous cell carcinoma of larynx was the same in different ages and genders (23).

A significant relationship between lymph node involvement and Ki67 positive cells counts was found. This is compatible with other reports (23-24).

This finding was not compatible with Sommer and Olofsson report. They demonstrated that no correlation exist between ki67 expression and lymph nodes involvement, tumor size and degree of differentiation (25).

The Sommer and Olofsson report completed in 1997.This controversy could be due to improvement the methods and technique from 1997 until recent years.

Studies have been reported that ki67 expression has a positive correlation with p53, VEGF and AgNOR expression (12,23-26).

This indicates that cellular proliferation in coordinate with apoptosis and angiogenesis improve malignancy.

Based on our review, this is the first study on the relationship between lymph node involvement with Ki67 expression and eosinophil cells infiltration in oral squamus cell carcinoma. There was a significant positive correlation between Ki67 expression and eosinophil cells counts.

The eosinophils and Ki67 positive cells count correlated to lymph node metastasis with 100%,100% sensitivity and 100%, 91.9% specificity, respectively. We belived that cellular proliferation and immune system are involved in tumor progression. Considering the obtained sensitivity and specificity, it seems that evaluating the eosinophils count and Ki67 expression in small specimen or ones with inadequate margins can be a good predictor of lymph node metastasis. Eosinophils count is also helpful for analyzing the lymph node in-

volvement on hematoxilin-eosin sections when immunohistochemical technique is not available.

For further investigation analysis of the blood eosinophils count would be a good field for obtaining more comperhensive results about predictating lymph node metastases in oral squamous cell carcinoma.This is a simpler and easier way than counting the eosinophil cells on tumoral tissues.

Conclusion

A significant relationship between lymph node involvement with eosinophilc cells and Ki67 positive cells counts were existed. The eosinophils and Ki67 positive cells count correlated to lymph node metastasis with 100%, 100% sensitivity and 100%, 91.9% specificity, respectively.

Acknowledgements

The authors thank Vaziri S., Daavoodi H., Ahad Pour Sefidan M., for immunohistochemical and histochemical processing.

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