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This article is being republished as the authors missed including some references and their citations in the earlier version.

Original Article

Quantitative assessment of tumor-associated tissue eosinophilia and mast cells in tumor proper and lymph nodes of oral squamous cell carcinoma

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is the most common cancer of the oral cavity. Tumor stage, thickness, lymph node metastasis (LNM), extranodal spread, perineural invasion, tumor differentiation, mutations, human papillomavirus infection and tumor microenvironment are independent prognostic indicators of OSCC. However, clinically, among all factors, LNM is considered an important prognostic factor in OSCC as it not only determines the stage of disease but also the strongest independent factor which predicts recurrence of disease. Further research proves that there are several biologically important factors in tumor tissue and LNs which promote or defend LNM. While it is proposed that tumor-associated tissue eosinophils (TATE) and mast cells (MCs) have "immuno-protective" effect, this remains unproven and various researchers have conflicting opinion.

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Aim: The aim is to determine the presence of TATE and MCs in OSCC and to evaluate if any association exists between them and LNM.

Study Design: It is a comparative retrospective study between two groups including 35 OSCC cases positive and 35 negative for LNM.

Materials and Methodology: Quantification of cells was done by counting total number of cells in 10 high-power fields under ×40 objective lens using "zigzag" method and dividing it by total number of fields. Eosinophils stained bright red with carbol chromotrope and MCs purple-violet with toluidine blue.

Statistics: Independent *t*-test and Pearson's correlation were done using STATA IC 0.2 software. The level of significance was at 5%. Comparison of eosinophil and MC infiltration was done based on gender, metastatic, nonmetastatic LN and in tumor proper.

Results and Conclusion: Our study showed weak positive correlation between mean eosinophils count in tumor and LNs which implies a definite association between the microenvironment of tumor, its progression and LNM. There was a significant association between MC density and decreased LNM also. We conclude that an increased number of immunological cells (TATE and MCs) are a favorable prognostic indicator in OSCC. There is evidence of reduction in LNM with increasing density of these immunological cells. Recognition of TATE and MCs as integral to tumor biology opens an avenue for novel approaches to cancer therapies.

Keywords: Lymph nodes, mast cells, tumor-associated tissue eosinophils

INTRODUCTION

Oral squamous cell carcinoma (OSCC), although seen worldwide, is more common in India. Lymph node (LN) metastasis is shown to be a strong prognostic factor in OSCC. Many histopathological and immunohistochemical markers have been studied to predict LN metastasis.[1]

OSCC is graded by degree of differentiation and amount of keratinization. Tumor microenvironment comprises a range of inflammatory cells, except neoplastic cells. These are chiefly lymphocytes, macrophages, neutrophils, plasma cell, mast cells (MCs) and eosinophils. Stromal response to tumor is characterized by intensity of lymphoplasmacytic infiltration around tumor, and dense lymphoplasmacytic invasion is presumably indicative of good host response to tumor. The role of MCs and eosinophils has been implicated in biology of tumors. Eosinophils are thought to become active following action of MCs which secrete histamine and ECF (eosinophils chemo attractant factor) and attract eosinophils in tissues.[2]

Tissue eosinophilia is a regular finding in allergic and parasitic disorders, but their role still needs to be evaluated in SCCs.[3] Eosinophils are present in large numbers in some SCC of the oral cavity, cervix, lower colon and anus.[4] Eosinophils release chemical substances under diverse stimuli, such as interleukins, chemokines (RANTES, endotoxin-1), eosinophil chemoattractant protein, major basic protein, EPO and EDN.[5] These substances may induce inflammation and cell death and contribute to tumor microenvironment. MCs play a diverse role that may contribute to defense against tumors or tumor progression. Recent studies have shown an increase in MC density (MCD) in OSCC, being associated with tumor-favoring effects.[6] Moreover, in an experimental model of carcinogenesis, MCD was associated with carcinoma development by upregulation of angiogenesis.[7]

While it is proposed that they have an "immuno-protective" effect, this remains unproven. Contradictory reports may relate to inconsistencies in counting. Hence, this study is taken up to evaluate infiltration of these immunological cells (eosinophils and MCs) in OSCC.

Cervical LN metastasis is a major factor of outcome in OSCC. Although histologic evaluation of invasiveness provides useful information, histopathologic diagnosis provides only partial information on neoplastic changes. Consequently, biomarkers which characterize tumor behavior and predict outcome have been sought to enhance treatment planning in patients with OSCC.[8]

Although only few studies have been done to understand the role of tumor-associated tissue eosinophils (TATE) and MCs in OSCC, it still remains unclear. It is further obscured by studies showing TATE and MCs associated with an improved prognosis and also with poor prognosis. The need for study is to determine the presence of TATE and MCs in OSCC and to evaluate if any relation exists between TATE and MCs with LN metastasis.

MATERIALS AND METHODOLOGY

The study was approved by the Institutional Ethical Committee. It was a comparative retrospective study of

12-month duration. Criteria for sample selection were to include histopathologically diagnosed OSCC cases which were surgically excised with concomitant neck dissections and to exclude patients with known primary other than oral cavity. Using 95% confidence interval and 80% power (Mann–Whitney test), formalin-fixed, paraffin-embedded tissue blocks of 70 OSCC resection cases with concomitant neck dissections, comprising 35 cases positive and 35 cases negative, for LN metastasis were retrieved from archives of the Department of Oral Pathology, MPDC, Vadodara.

Three sections of 4-μ thickness were made for each case using soft-tissue microtome and stained with hematoxylin and eosin, carbol chromotrope and toluidine blue stains, respectively.

Eosinophilic granules stained bright red with carbol chromotrope [Figure 1] and MC granules stained purple-violet with toluidine blue [Figure 2]. These cells were then observed under compound microscope. Quantification was done by randomly selecting 10 high-power fields in each slide which showed high density of these cells. Each field was screened under ×40 objective lens using "zigzag" method for evaluation of TATE and MCs. Total numbers of cells were counted and divided by total number of fields to obtain an average number of cells. Cells were counted, data were tabulated and statistical analysis was done.

Statistical analysis

It was done with Stata IC version 13.0, (StataCorp LLC, Texas, USA) using independent *t*-test and Pearson's correlation. The level of significance was set at 5%. Comparison of eosinophil and MC infiltration was done based on gender, metastatic and nonmetastatic LNs and in tumor proper.

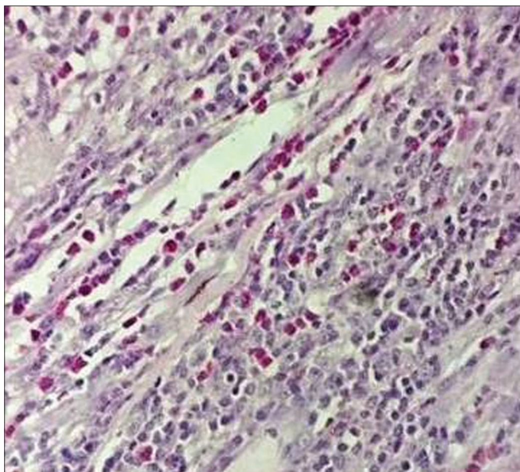

Figure 1: Eosinophils stained by carbol chromotrope in nonmetastatic lymph node (×400)

RESULTS

Among total metastatic cases, 77.1% (27) were male whereas 22.9% (8) were female. Among total nonmetastatic cases, 65.7% (23) were male whereas 34.3% (12) were female. There was no significant difference in gender distribution between two groups ($P = 0.290$).

Among metastatic cases, 40% (14) were from tongue, followed by 22.9% (8) from buccal mucosa, 11.4% (4) from lower alveolus, 8.6% (3) from palate, 5.7% (2) from lower sulcus and 2.9% (1) each from corner of mouth, floor of mouth, maxilla and retromolar triangle. Among nonmetastatic cases, 51.4% (18) were from tongue, followed by 25.7% (9) from buccal mucosa, 8.6% (3) from lower alveolus, 5.7% (2) each from floor of the mouth and retromolar triangle and 2.9% (1) from lower lip.

Mean age among metastatic group was 50.06 ± 13.86 whereas among nonmetastatic group was 49.06 ± 11.30. There was no significant difference in mean age between two groups ($P = 0.742$).

Mean eosinophil count in tumor proper of metastatic group was 4.03 ± 3.05 and of nonmetastatic group was 8.71 ± 4.40. There was a significant difference in mean eosinophil count in tumor proper between two groups ($P < 0.001$) [Tables 1a and b].

Table 1a: Distribution of tumor-associated tissue eosinophils between metastatic and nonmetastatic groups (independent *t*-test)

	Group	*n*	Mean	SD	SEM
Mean number of TATE in primary tumor	Metastatic	35	4.0314	3.05545	0.51646
	Nonmetastatic	35	8.7143	4.40048	0.74382
Mean number of TATE in lymph nodes	Metastatic	35	5.0600	3.97079	0.67119
	Nonmetastatic	35	7.7629	3.74198	0.63251

TATE: Tumor-associated tissue eosinophils, SD: Standard deviation, SEM: Standard error of mean

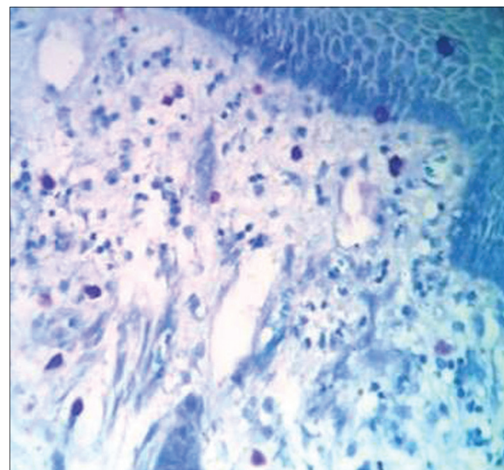

Figure 2: Mast cells stained by toluidine blue in tumor stroma (×400)

Table 1b: Evaluation of *P* value

	t	df	*P*	Mean difference	SE difference	95% CI of the difference	
						Lower	Upper
Mean number of TATE in primary tumor	−5.171	68	<0.001	−4.68286	0.90554	−6.48983	−2.87588
Mean number of TATE in lymph nodes	−2.931	68	0.005	−2.70286	0.92226	−4.54319	−0.86252

CI: Confidence interval, SE: Standard error, TATE: Tumor-associated tissue eosinophils

Mean eosinophil count in LNs of metastatic group was 5.06 ± 3.97 and nonmetastatic group was 7.76 ± 3.74. There was a significant difference in mean eosinophil count in LNs between two groups ($P = 0.005$) [Tables 1a and b].

Relationship between mean eosinophil count in tumor and mean eosinophil count in LNs suggest a weak positive correlation. The correlation value was 0.234 for metastatic group and 0.067 for nonmetastatic group [Tables 2a and b].

Mean MC count in tumor proper of metastatic group was 6.91 ± 3.84 and of nonmetastatic group was 8.48 ± 2.83. There was no significant difference in mean MC count in tumor proper between two groups ($P = 0.056$) [Tables 3a and b].

Mean MC count in LNs of metastatic group was 4.17 ± 2.89 and nonmetastatic group was 5.81 ± 3.41. There was a significant association in mean MC count in LNs between two groups ($P = 0.034$) [Tables 3a and b].

The relationship between mean number of MCs in tumor and mean number of MCs in LNs suggest a weak positive correlation. The correlation value was 0.237 for metastatic group and 0.091 for nonmetastatic group [Tables 4a and b].

DISCUSSION

Cancer terminates thousands of lives daily. Despite numerous attempts to find a permanent cure, overall survival rate has not increased, reason being restricted knowledge of tumor microenvironment. Therefore, this study was done in search of new prognostic markers for OSCC.

Tissue eosinophilia in SCC has long been known; however, its role in tumor development remains debatable. Studies have reported both favorable and unfavorable prognoses for patients with tumors exhibiting TATE. Eosinophil infiltration in malignant tumor is seen in variety of tissues. It does not relate to site or etiology of tumor, nor to an idiosyncrasy of patients in whom it occurs.[9]

Various studies exist determining the role of TATE in carcinomas of nasopharynx, esophagus, breast, stomach and cervix. However, very few have been done in OSCC.

Table 2a: Correlation of distribution of mean eosinophil count in primary tumor and in lymph nodes (group=metastatic)

Pearson correlation	0.234
P	0.176
n	35

Table 2b: Correlation of distribution of mean eosinophil count in primary tumor and in lymph nodes (group=nonmetastatic)

Pearson correlation	0.067
P	0.703
n	35

Moreover, various parameters have been researched in relation to TATE including tumor size and distant metastasis. However, not many studies relate TATE with LN metastasis.

Results of the present study show no statistical significance in relation to site. Among metastatic cases, 40% were from tongue, followed by 22.9% from buccal mucosa, 11.4% from lower alveolus, 8.6% from palate, 5.7% from lower sulcus and 2.9% each from corner of the mouth, floor of mouth, maxilla and retromolar triangle. Among nonmetastatic cases, 51.4% were from tongue, followed by 25.7% from buccal mucosa, 8.6% from lower alveolus, 5.7% each from floor of the mouth and retromolar triangle and 2.9% from the lower lip. This goes in concordance with studies done by Dorta *et al.* and Oliveira *et al.* who found no statistically significant differences in distribution of eosinophils among these sites.[10,11]

Our study suggested a weak positive correlation between mean eosinophil count in tumor and in LNs. Correlation value is 0.234 for metastatic group and 0.067 for nonmetastatic group. Contrary to this, Looi (1987) observed that TATE in primary tumor was not always associated with eosinophilia in the metastases.[12]

Our study also demonstrates a higher count of TATE in nonmetastatic cases of OSCC when compared to metastatic cases, which is in concordance with studies done by Ohashi *et al.* and Ishibashi *et al.* Findings indicate the cruciality of TATE in biological behavior of OSCC. The number of tumor-associated eosinophils was significantly higher in cases without LN metastasis. This suggests possible correlation between TATE and a less aggressive biological behavior of tumor.[3,13]

Table 3a: Distribution of mast cells between metastatic and nonmetastatic groups (independent *t*-test)

	Group	*n*	Mean	SD	SEM
Mean number of MCs in primary tumor	Metastatic	35	6.911429	3.8480531	0.6504397
	Nonmetastatic	35	8.485714	2.8360228	0.4793753
Mean number of MCs in lymph node	Metastatic	35	4.177143	2.8932056	0.4890410
	Nonmetastatic	35	5.814286	3.4126187	0.5768378

MCs: Mast cells, SD: Standard deviation, SEM: Standard error of mean

Table 3b: Evaluation of *P* value

	t	df	*P*	Mean difference	SE difference	95% CI of the difference	
						Lower	Upper
Mean number of MCs in primary tumor	−1.948	68	0.056	−1.5742857	0.8080053	−3.1866351	0.0380637
	−1.948	62.521	0.056	−1.5742857	0.8080053	−3.1891975	0.0406261
Mean number of MCs in lymph node	−2.165	68	0.034	−1.6371429	0.7562427	−3.1462016	−0.1280841
	−2.165	66.227	0.034	−1.6371429	0.7562427	−3.1469336	−0.1273521

CI: Confidence interval, SE: Standard error, MCs: Mast cells

Table 4a: Correlation of distribution of mean mast cell count in primary tumor and in lymph nodes (group=metastatic)

Pearson correlation	0.237
P	0.171
n	35

Table 4b: Correlation of distribution of mean mast cell count in primary tumor and in lymph nodes (group=nonmetastatic)

Pearson correlation	0.091
P	0.603
n	35

Regarding the role of TATE various studies were conducted by Goldsmith *et al*. (1987), Iwasaki *et al*. (1986) and Debta *et al*. (2011).[14-16]All these studies suggest that increased number of TATE is associated with antitumoral role and shows good prognosis. These are in concordance with our study which also shows an increase in TATE in nonmetastatic cases than metastatic cases, hence favoring good prognosis. Mean eosinophil count in tumor proper of metastatic group is 4.03 ± 3.05 and nonmetastatic group is 8.71 ± 4.40. Thus, there is statistically significant difference in mean eosinophil count in tumor proper between two groups ($P < 0.001$). Mean eosinophil count in LNs of metastatic group is 5.06 ± 3.97 and nonmetastatic group is 7.76 ± 3.74. There is also a significant difference in mean eosinophil count in LNs between two groups ($P = 0.005$).

However, many other studies suggest a tumor-promoting role of eosinophils like those done by David *et al*. (1981), van Driel *et al*., Wong *et al*. and Alrawi *et al*.[9,17-19]These studies are in contrast with present study which suggests a favorable prognostic implication of TATE. These studies suggest that an elevated TATE in SCC is associated with aggressive tumor biology and presence of higher number of eosinophils in an excisional specimen should indicate the need for additional therapeutic measures and close surveillance to detect earlier locoregional recurrence and possible distant metastasis.

MCs play a role in tumor microenvironment, and increased MCD has been demonstrated in OSCC. Serum tryptase levels are elevated with some malignant tumors and may thus be a useful parameter. However, there are no data available about OSCC.[20] MCs are found to accumulate around and within many types of solid cancers, and recently, MC function in developing tumors have been extensively reviewed, with varying suggestions that they may shift the balance either in favor of or against tumor growth.[21] A study done by Tanooka *et al*. in mice, support the hypothesis that MCs are involved in tumor suppression. Another study on the effects of long-term administration of cancer-promoting substances on oral subepithelial MCs in rat done by Sand *et al*. suggests that MCs play a role in immunological cell defense against chemical carcinogens.[22,23]

In a study done by Tomita *et al*., MC count was significantly higher in nonmetastatic nodes than in metastatic nodes. The same observation was noted in our study with an increase in MC count in nonmetastatic LNs when compared to metastatic nodes. Mean MC count in LNs of metastatic group was 4.17 ± 2.89 and nonmetastatic group was 5.81 ± 3.41. There was a significant association in mean MC count in LNs between two groups ($P = 0.034$).[24]

Dabiri *et al*. showed that the presence of MCs in peritumoral stroma correlates with a good prognosis in breast cancers with a long-term follow-up, supporting an important role for host MCs in breast cancer. This goes in concordance with present study where mean MC count in tumor proper of metastatic group is 6.91 ± 3.84 and of nonmetastatic group is 8.48 ± 2.83 ($P = 0.056$).[25]

The purpose of a study done by Samoszuk *et al*. was to test the hypothesis that MCs present in fibrotic regions of cancer can suppress the growth of tumor cells through

an indirect mechanism involving peritumoral fibroblasts. Degranulating MCs are restricted to peritumoral fibrous tissue, and MC heparin is a powerful inhibitor of clonogenic growth of tumor cells co-cultured with fibroblasts. These results may help to explain the well-known ability of heparin to inhibit the growth of primary and metastatic tumors.[26]

Various studies done by Tanooka *et al*., Sand *et al*., Tomita *et al*., Dabiri *et al*., Samoszuk *et al*., Alkhabuli and High (2006), Sinnamon *et al*., Debta *et al*. and Divyarani *et al*. (2014) suggested an inverse relationship between number of MCs and the amount of tumor tissue.[16,22-29] They are suggestive of antitumoral role of MCs and its correlation with good prognosis. Our study is in concordance with these studies and suggests MCs to favor good prognosis. Relationship between mean number of MCs in tumor and mean number of MCs in LNs suggest a weak positive correlation in the present study. Correlation value is 0.237 for metastatic group and 0.091 for nonmetastatic group.

However, various other studies suggest that MCs play a tumor-promoting role. These studies done by Yano *et al*., Imada *et al*., by Elpek *et al*., Iamaroon *et al*., Rojas *et al*., Madhuri Ankle *et al*., Fakhrjou *et al*. (2014) and A Anuradha *et al*. are in contrast to the present study which shows higher MC count in nonmetastatic cases, both in tumor proper and in LNs, suggesting a negative role of MCs in tumor growth and metastasis.[6,7,30-35]

CONCLUSION

Recognition of TATE and MCs as integral to tumor growth opens an avenue for novel approaches to cancer therapies to decrease tumor growth and metastasis.[21] We conclude that both immunological cells (TATE and MCs) have an effect on OSCC. Thus, quantitative assessment of these cells is important aspects of microscopic OSCC evaluation. For proper evaluation of these cells, special stains are an important tool that are budget-friendly and give an acceptable rapid result. With results of our study, we conclude that an increased number of TATE and MCs were found to be a favorable prognostic indicator in OSCC with an increased mean cell count observed in nonmetastatic OSCC cases when compared to metastatic cases. A decrease in these cells possibly reflects an important modification in microenvironment during tumor initiation and progression. Currently, exact functional relevance of these cells in tumors is perplexing. Their role needs to be further validated using larger samples that include recurrent cases and follow-up studies. Hence, in search of new prognostic and predictive factors for OSCC, we conclude that an increase in infiltration of TATE and MCs is associated with favorable prognosis in OSCC. Thus, quantitative assessment of eosinophils and MCs is the most essential aspects of microscopic evaluation of OSCC.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Suresh TN, Hemalatha A, Harendra Kumar ML, Azeem Mohiyuddin SM. Evaluation of histomorphological and immunohistochemical parameters as biomarkers of cervical lymph node metastasis in squamous cell carcinoma of oral cavity: A retrospective study. J Oral Maxillofac Pathol 2015;19:18-24.
2. Culling CF, Allison RT, Barr WT. Cellular Pathology Technique. 4th ed. London: Butterworth and Co., Ltd.; 1985. p. 460-1.
3. Ohashi Y, Ishibashi S, Suzuki T, Shineha R, Moriya T, Satomi S, *et al*. Significance of tumor associated tissue eosinophilia and other inflammatory cell infiltrate in early esophageal squamous cell carcinoma. Anticancer Res 2000;20:3025-30.
4. Lorena SC, Dorta RG, Landman G, Nonogaki S, Oliveira DT. Morphometric analysis of the tumor associated tissue eosinophilia in the oral squamous cell carcinoma using different staining techniques. Histol Histopathol 2003;18:709-13.
5. Rothenberg ME, Hogan SP. The eosinophil. Annu Rev Immunol 2006;24:147-74.
6. Iamaroon A, Pongsiriwet S, Jittidecharaks S, Pattanaporn K, Prapayasatok S, Wanachantararak S, *et al*. Increase of mast cells and tumor angiogenesis in oral squamous cell carcinoma. J Oral Pathol Med 2003;32:195-9.
7. Rojas IG, Spencer ML, Martínez A, Maurelia MA, Rudolph MI. Characterization of mast cell subpopulations in lip cancer. J Oral Pathol Med 2005;34:268-73.
8. Nagata M, Fujita H, Ida H, Hoshina H, Inoue T, Seki Y, *et al*. Identification of potential biomarkers of lymph node metastasis in oral squamous cell carcinoma by cDNA microarray analysis. Int J Cancer 2003;106:683-9.
9. David L, Johanna J, Hutt MS. Tumour-associated eosinophilia: A review. J Clin Pathol 1981;34:1343-8.
10. Oliveira DT, Biassi TP, Faustino SE, Carvalho AL, Landman G, Kowalski LP, *et al*. Eosinophils may predict occult lymph node metastasis in early oral cancer. Clin Oral Investig 2012;16:1523-8.
11. Dorta RG, Landman G, Kowalski LP, Lauris JR, Latorre MR, Oliveira DT, *et al*. Tumour-associated tissue eosinophilia as a prognostic factor in oral squamous cell carcinomas. Histopathology 2002;41:152-7.
12. Looi LM. Tumor-associated tissue eosinophilia in nasopharyngeal carcinoma. A pathologic study of 422 primary and 138 metastatic tumors. Cancer 1987;59:466-70.
13. Ishibashi S, Ohashi Y, Suzuki T, Miyazaki S, Moriya T, Satomi S, *et al*. Tumor-associated tissue eosinophilia in human esophageal squamous cell carcinoma. Anticancer Res 2006;26:1419-24.
14. Goldsmith MM, Cresson DH, Askin FB. The prognostic significance of stromal eosinophilia in head and neck cancer. Otolaryngol Head Neck Surg 1987;96:319-24.
15. Iwasaki K, Torisu M, Fujimura T. Malignant tumor and eosinophils. I. Prognostic significance in gastric cancer. Cancer 1986;58:1321-7.
16. Debta P, Debta FM, Vijay Wadhwan MC. Evaluation of prognostic significance of immunological cells (tissue eosinophil and mast cell) infiltration in oral squamous cell carcinoma. J Cancer Sci Ther

2011;3:201-4.

17. van Driel WJ, Hogendoorn PC, Jansen FW, Zwinderman AH, Trimbos JB, Fleuren GJ, *et al*. Tumor-associated eosinophilic infiltrate of cervical cancer is indicative for a less effective immune response. Hum Pathol 1996;27:904-11.
18. Wong DT, Bowen SM, Elovic A, Gallagher GT, Weller PF. Eosinophil ablation and tumor development. Oral Oncol 1999;35:496-501.
19. Alrawi SJ, Tan D, Stoler DL, Dayton M, Anderson GR, Mojica P, *et al*. Tissue eosinophilic infiltration: A useful marker for assessing stromal invasion, survival and locoregional recurrence in head and neck squamous neoplasia. Cancer J 2005;11:217-25.
20. Jaafari-Ashkavandi Z, Khademi B, Akbari S, Malekzadeh M. Serum level of mast cell tryptase in patients with oral squamous cell carcinoma: Lack of correlation with clinicopathologic factors. Asian Pac J Cancer Prev 2013;14:2955-8.
21. Maltby S, Khazaie K, McNagny KM. Mast cells in tumor growth: Angiogenesis, tissue remodelling and immune-modulation. Biochim Biophys Acta 2009;1796:19-26.
22. Tanooka H, Kitamura Y, Sado T, Tanaka K, Nagase M, Kondo S, *et al*. Evidence for involvement of mast cells in tumor suppression in mice. J Natl Cancer Inst 1982;69:1305-9.
23. Sand L, Hilliges M, Larsson PA, Wallstrom M, Hirsch JM. Effects of long-term administration of cancer-promoting substances on oral subepithelial mast cells in the rat. Anticancer Res 2002;22:2623-7.
24. Tomita M, Matsuzaki Y, Edagawa M, Shimizu T, Hara M, Onitsuka T, *et al*. Distribution of mast cells in mediastinal lymph nodes from lung cancer patients. World J Surg Oncol 2003;1:25.
25. Dabiri S, Huntsman D, Makretsov N, Cheang M, Gilks B, Bajdik C, *et al*. The presence of stromal mast cells identifies a subset of invasive breast cancers with a favorable prognosis. Mod Pathol 2004;17:690-5.
26. Samoszuk M, Kanakubo E, Chan JK. Degranulating mast cells in fibrotic regions of human tumors and evidence that mast cell heparin interferes with the growth of tumor cells through a mechanism involving fibroblasts. BMC Cancer 2005;5:121.
27. Alkhabuli JO, High AS. Significance of eosinophil counting in tumor associated tissue eosinophilia (TATE). Oral Oncol 2006;42:849-50.
28. Sinnamon MJ, Carter KJ, Sims LP, Lafleur B, Fingleton B, Matrisian LM, *et al*. A protective role of mast cells in intestinal tumorigenesis. Carcinogenesis 2008;29:880-6.
29. Divyarani MN, Harendra Kumar ML, Swaroop Raj BV, Manoranjan UD. Quantitative analysis of mast cells in invasive ductal carcinoma. Int J Curr Res Acad Rev 2014;2:91-6.
30. Yano H, Kinuta M, Tateishi H, Nakano Y, Matsui S, Monden T, *et al*. Mast cell infiltration around gastric cancer cells correlates with tumor angiogenesis and metastasis. Gastric Cancer 1999;2:26-32.
31. Imada A, Shijubo N, Kojima H, Abe S. Mast cells correlate with angiogenesis and poor outcome in stage I lung adenocarcinoma. Eur Respir J 2000;15:1087-93.
32. Elpek GO, Gelen T, Aksoy NH, Erdoğan A, Dertsiz L, Demircan A, *et al*. The prognostic relevance of angiogenesis and mast cells in squamous cell carcinoma of the oesophagus. J Clin Pathol 2001;54:940-4.
33. Madhuri Ankle R, Alka Kale D, Nayak R. Mast cells are increased in leukoplakia, oral submucous fibrosis, oral lichen planus and oral squamous cell carcinoma. J Oral Maxillofac Pathol 2017;11:18-22.
34. Fakhrjou A, Niroumand-Oscoei SM, Somi MH, Ghojazadeh M, Naghashi S, Samankan S, *et al*. Prognostic value of tumor-infiltrating mast cells in outcome of patients with esophagus squamous cell carcinoma. J Gastrointest Cancer 2014;45:48-53.
35. Anuradha A, Kiran Kumar Naik B, Vijay Srinivas G, Devi RS, Puneet HK. Incidence of mast cells in oral squamous cell carcinoma: A short study. J Oncol 2014;2014:614291.