Quantitative analysis of tumor-associated tissue eosinophils and tumor-associated blood eosinophils in oral squamous cell carcinoma

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Abstract

**Background:** Stromal response to cancer is usually characterized by intense lymphoplasmacytic infiltrate. However, recently, the attention has shifted to tumor-associated tissue eosinophils (TATE). Tumor-associated blood eosinophils (TABE) are rare in solid cancers; however, carcinoma of the head and neck shows its prevalence.

**Aim:** The aim of the study was to investigate the prevalence and relationship of tissue and blood eosinophils in various grades of oral cancer. The purpose of the article is to emphasize the possible clinical and biological significance of eosinophils in patients of oral squamous cell carcinoma (OSCC) so that appropriate therapeutic strategies can be devised accordingly.

**Study Design:** Thirty histologically confirmed cases of oral squamous cell carcinoma were divided into well, moderate and poorly differentiated carcinoma. Eosinophilic infiltration in the tissue was graded as low, moderate and massive TATE. The number of eosinophils per 100 WBCs was taken as the differential eosinophil count. Blood eosinophilia (BE) >6% was considered to be TABE.

**Materials and Methods:** Hematoxylin and eosin-stained tissue sections at 5 µ were evaluated. Prolonged staining in dilute 0.05% aqueous eosin demonstrated eosinophils selectively. Blood smears were stained by Leishman stain.

**Statistical Analysis:** Student’s t-test, Chi-square test, ANOVA, Newman–Keuls Multiple Comparison Test and Karl Pearson correlation coefficient® method were used.

**Results:** The mean TATE value was highest in poorly differentiated carcinoma. TABE was seen only in a few cases and was associated mostly with poorly differentiated OSCC.

**Conclusion:** There was a statistically significant correlation between TATE and histological grades of OSCC. Eosinophilia of the peripheral blood is an adverse sign in patients with carcinoma.

**Keywords:** Eosinophils, oral cancer, oral squamous cell carcinoma, tumor-associated blood eosinophilia, tumor-associated tissue eosinophilia
INTRODUCTION

Oral cancer is one of the ten most common cancers in the world.\(^1\) Half of the patients affected with the disease die within the first 2 years of diagnosis. The World Health Organization predicts a continuing worldwide increase in the incidence of oral cancer.\(^2\)

Cancer comes into existence by the creation of abnormal cells that grow beyond their natural boundaries.\(^3\) Incipient cancer cells acquire traits that enable them to become tumorigenic and ultimately malignant.\(^4\) However, the formation of a clinically relevant tumor also requires the support of the surrounding normal stroma, known as the tumor microenvironment. The tumor microenvironment contains many distinct cell types including the immune cells.\(^5\) Some of these cells support neoplastic growth, whereas others are involved in killing of the tumor.\(^6\) Emerging evidence indicates that to effectively control cancer, we need to target both the tumor cells and other cells present in the microenvironment.\(^7\)

Oral squamous cell carcinoma (OSCC) at some stage is associated with chronic inflammation in the adjacent connective tissue.\(^7\) This stromal response is usually characterized by intense lymphoplasmacytic infiltrate. Little attention has been given to the presence of eosinophils in the stroma of OSCC. Recent findings indicate that eosinophils have a potential role in oncology.\(^6\,8\)

Eosinophil was first observed by Wharton Jones in 1846 was so named by Paul Ehrlich in 1879.\(^9\) They are bone marrow-derived, terminally differentiated granulocytes that normally circulate in the blood in low numbers and tend to localize in those tissues with mucosal epithelial surfaces.\(^10\)

They comprise about 1%–3% of white blood cells in the blood and are 40–450 cells/mm\(^3\). The diameter is 10–12 \(\mu\) and the nucleus is in the shape of two lobes connected by a bridge. They are characterized by red-stained granules in their cytoplasm.\(^11\) They produce an arsenal of enzymes and lipid mediators, which are implicated in its effector functions.\(^9\)

Tumor-associated tissue eosinophilia (TATE) is defined as the stromal infiltration of a tumor by eosinophils and was first described by Przewoski in 1896 in carcinoma of the cervix. The phenomenon has been recognized in a variety of carcinomas, mainly tumors at a body surface.\(^12\) Quantification of TATE can be easily performed on routine hematoxylin and eosin (H and E) stained slides and does not warrant specific immunohistochemical analysis.\(^13\)

Tumor-associated blood eosinophils (TABE) was first described in 1893.\(^14\) It occurs most frequently in hematologic malignancies and is rare in solid cancers. Carcinomas of the head and neck, ovary, uterus, breast, pancreas, lung, liver, thyroid gland and (GI) tract exhibit TABE.\(^15\)

The aim of the study was to investigate the prevalence, relationship and significance of tissue and blood eosinophils in various grades of OSCC.

MATERIALS AND METHODS

The study was undertaken after approval from the institutional ethical committee. From the year 2011–2014, patients with OSCC were screened. Patients with other coexisting primary tumors and unresectable tumors and those subjected to radiotherapy, chemotherapy or other treatment prior to surgery were excluded from the study. Patients with a history of diseases interfering with white blood cell counts, bone marrow diseases, respiratory system failure, rheumatologic diseases, allergy, asthma, dermatitis and active infectious diseases in recent months and known cases of parasitic infections were also excluded from the study.

Written consent from each patient was obtained. The study was divided into two groups. Group I, the control group, consisted of 30 cases in which biopsy samples of the normal healthy oral mucosa (taken from patients with an impacted third molar and with no signs of inflammation in the overlying mucosa at the time of removal) were chosen. Assessment of the impacted tooth was done by physical and radiographic evaluation. Blood samples of the same patient (drawn for routine blood investigations prior to operculectomy) were included in the study. In the study group, i.e., Group II, cases of OSCC histologically confirmed on biopsy and with no evidence of tumor ulceration and necrosis were chosen for the study. Blood samples of the same patient (drawn for routine blood investigations prior to biopsy) were taken.

Proper clinical data including standard demographic data (age, gender, occupation and habits) were recorded. A thorough clinical examination was done including the site and size of the lesion, nodal status and metastasis. Tissue obtained by excisional biopsy was fixed in 10% neutral buffer formalin. Two milliliter of intravenous blood was collected from the patients following aseptic precautions in an ethylenediamine tetraacetic acid vial.

The samples in Group II were further divided into three groups as well, moderate and poorly differentiated carcinoma.
Eosinophilic infiltration in the tissue was evaluated on 5 μm H and E-stained tissue sections. Prolonged staining in dilute 0.05% aqueous eosin demonstrates eosinophils selectively. A number of eosinophils per high-power field (HPF) were counted using a light microscope (Olympus BX 51). One HPF is defined by a ×10 eyepiece and a ×40 objective. TATE was considered positive when eosinophils were >10/HPF. Less than 10 HPF were considered as low infiltration. TATE was graded as follows:

- >10 but <100 eosinophils/HPFs in 10 fields = moderate TATE
- >100 eosinophils/HPF = massive TATE

In the control group, low eosinophilic infiltration was seen in 21 (70%) cases, whereas it was absent in 9 (30%) cases. In the study group, eosinophils were present in all the 30 cases (100%). The maximum number of cases, 18 cases (60%), showed low eosinophilic infiltration, 11 cases (36.6%) showed moderate TATE, whereas only 1 case (3.33%) showed massive eosinophilia.

The tissue eosinophil (TE) values of the control and study groups ranged from 0.0–1.2 to 1.0–103.2, respectively, with mean ± standard deviation of 0.17 ± 0.24 and 21.59 ± 33.13, respectively. The mean TE of the study group was comparatively higher than the control group. Comparing the mean TE of two groups, t-test revealed significantly different and higher (99.2%) eosinophils in the study group ($t = 9.68; P < 0.001$).

TE values increased as the degree of dedifferentiation increases in OSCC as shown in Table 1 and Figures 1-3. Comparing the mean TE levels of four groups, ANOVA revealed significantly different levels among the groups ($F = 314.03, P < 0.001$).

In the control group, in none of the cases, BE levels were >6%, whereas TABE was seen in 23.3% cases of the study group. Among the OSCC group, there were none of the well-differentiated carcinoma cases, few cases of moderately differentiated carcinoma, whereas all the cases of poorly differentiated carcinomas exhibited TABE, as shown in Table 3 and Figure 4.

Correlation analysis revealed a significant and positive (direct) correlation between TE and BE ($r = 0.90, P < 0.001$). Further, regression analysis showed that the BE can be estimated significantly by TE with a high coefficient of determination ($R^2$) 81.0%.

**RESULTS**

In our study, most of the cases belonged to the older age group reinforcing the findings of other authors who also suggested that oral cancer typically occurs in the elderly. Most of the individuals were males, probably because of the predominance of risk factors in males. The most prevalent habit was that of tobacco chewing and the most commonly affected site was the BM. Tobacco chewers frequently place tobacco and betel quid in the buccal vestibule compressed against the BM, which acts as a source of continuous irritation to this site.

TE values ranged from 1 to 103 in the study group. This variation in values can be attributed to individual variation in the host immune response to the tumor. The mean TE values in the study group were found to be significantly higher ($P < 0.05$) as compared to the control group, suggesting that more eosinophils have infiltrated carcinoma tissues as compared to the normal oral mucosa. This was in accordance with other studies which found higher eosinophil count in the carcinoma group.

Regarding the prevalence of TATE in OSCC, it has been seen that TATE is seen in certain tumors only such as carcinoma of the vagina, penis, skin, nasopharynx, larynx, GI tract, lung and oral cavity. There is little in common between them, except that all are tumors that occur at a body surface. Other than Hodgkin’s disease, TATE is rarely seen in sarcomas.

The mean TE values were higher in poorly differentiated carcinomas as compared to moderately carcinoma and...
marked eosinophilic infiltration was highly chemotactic for eosinophils in vitro. Small molecules released from stressed/dying cells, for example, damage-associated molecular pattern molecules act as chemoattractants and cause accumulation of eosinophils within the tissue. Therefore, carcinomas with increased areas of necrosis and cell death show increased accumulation. Eotaxin, lymphocyte-derived interleukins (ILs), regulated on activation normal T-cell expressed and secreted, platelet-activating factor (PAF) and 5-oxoeicosanoids are other factors that cause eosinophil accumulation. This could explain why a maximum number of eosinophils were seen in poorly differentiated carcinoma in our study.
The tissue microenvironment in which eosinophils accumulate supplies the necessary survival and differentiation factors for these accumulating eosinophils. However, contrary to our findings, few studies found no statistically significant relationship between TATE and histological grades of head-and-neck carcinoma in their study. 

Regarding its role in carcinoma, it has been seen that both the presence and state of activation of immunological cells play a role in the progression of the tumor. Tumor-associated eosinophils have at least two dominant nonoverlapping functions. One of these is limiting tumor growth and causing recruitment and activation of other leukocytes. The second is promoting tumor proliferation by immunoregulating and remodeling activity and by suppressing immune response.

Eosinophils kill tumor cells by degranulating and liberating their highly cytotoxic cationic proteins. Eosinophils may effect direct or antibody-dependent cancer lysis or they may interact with CD4+ lymphocytes by serving as antigen-presenting cells. They also collaborate with other inflammatory cells such as CD68+ macrophages and CD8+ T-cells to yield a better clinical outcome.

Eosinophils are also capable of downregulating the antitumor immunity, mainly through IL-10 and indoleamine (IDO) production. IL10 is a potent inhibitor of MHC complex and suppresses dendritic cell differentiation. IDO causes apoptosis of CD4 T-cells thus plays a role in escape from immune surveillance.

Eosinophils produce vascular endothelial growth factor, fibroblast growth factor, tumor necrosis factor (TNF)-α, granulocyte macrophage–colony-stimulating factor (GM-CSF), nerve growth factor, transforming growth factor-β and IL-8; these promote angiogenesis, remodel collagen fibers and suppress the immune system.

Neither of the two roles are mutually exclusive of each other. The final role that eosinophil plays is dictated by the tissue immune microenvironment where they accumulate. In a Th-2 polarized microenvironment, there is an exacerbation of local immune responses, whereas in a Th-1 polarized microenvironment, the suppression of immune response occurs.

Few studies have found TATE as a favorable prognostic indicator for squamous cell carcinoma of the head and neck supporting its antitumoral role, whereas in other studies, heavy eosinophilic infiltration was found to be associated with unfavorable prognosis, suggesting that TATE has a role in tumor progression. The ambiguities regarding the prognostic value of tumor-associated eosinophils are a reflection of this complex dual role. In some of the studies, a statistically significant association was observed between intense degree of TATE and locoregional recurrence.

We found the prevalence of TABE in 23.3% of cases of the study group. BE is associated with 5% of all malignant tumors and 5% to 33% of OSCCs. In one of the studies, blood eosinophils were evaluated in 157 patients of OSCC and TABE was present in 12.10% of them. In another study, it was found in 11.8% of OSCC cases.

Raised BE counts in OSCC patients as compared to normals can be attributed to generalized leukocytosis that occurs in malignancy, whereas BE >6% is an adverse sign in patients with carcinoma and is related to tumor progression. TABE is recognized as an indicator of tumor dissemination and generally related to poor prognosis.

Eosinophilia of the blood and bone marrow is more frequent and severe in aggressive tumors, especially when they have metastasized. Necrotic changes that occur within the tumor causes the release of eosinophilic proteins. Additional factors with similar functions include platelet-activating factor (PAF) and TNF. Seeding of metastases in the bone marrow also causes stimulation of eosinophil production.

### Table 2: Pairwise comparison of tissue eosinophil levels between the groups by Newman-Keuls test

| Comparisons                                      | Mean difference | P     |
|--------------------------------------------------|-----------------|-------|
| Normal versus well differentiated                | 3.17            | <0.001|
| Normal versus moderately differentiated          | 20.33           | <0.001|
| Normal versus poorly differentiated              | 92.05           | <0.001|
| Well differentiated versus moderately differentiated | 17.15           | <0.001|
| Well differentiated versus poorly differentiated  | 88.87           | <0.001|
| Moderately differentiated versus poorly differentiated | 71.72           | <0.001|

### Table 3: Tumor-associated blood eosinophils in various histological grades of oral squamous cell carcinoma

| Histological grades | Total number of subjects (n=30) | Number of subjects (%) showing TABE (BE >6%) | TABE values (mean±SD) |
|---------------------|---------------------------------|---------------------------------------------|-----------------------|
| Well differentiated  | 19                              | 0 (0)                                       | 0                     |
| Moderately differentiated | 6                              | 2 (33.3)                                   | 6.17±3.66             |
| Poorly differentiated | 5                              | 5 (100)                                    | 14±2.55               |

TABE: Tumor-associated blood eosinophils, BE: Blood eosinophilia, SD: Standard deviation
Production of eosinophils is under the control of GM-CSF and IL-3 and 5.[45] IL-3 and GM-CSF increases eosinophil production along with the other cells of different lineage. IL-5 supports exclusively eosinophil colony formation. GM-CSF prolongs the survival of eosinophils and enhances their cytotoxicity.[45] In metastatic deposits, GM-CSF is produced by stromal cells in the bone marrow.[39]

When an association was sought between TATE and TABE, it was seen that TABE was not always associated with TATE. There have been a large number of reports describing TATE, both with and without concomitant tumor associated blood eosinophilia (TABE). These two aspects of eosinophilia with tumors often show disparity and may represent quite different host responses.[23]

The limitation of our study was that because of a lack of a universal grading system, comparison of our data with other published data could not be done. Further studies are needed to emphasize the possible clinical and biological significance of eosinophils in patients of OSCC so that appropriate therapeutic strategies can be devised accordingly.

**CONCLUSION**

On the basis of our study, we conclude that there could be a close association between the low degree of tumor cell differentiation and strong eosinophilic infiltration. TABE could be considered an ominous sign more frequently seen in aggressive tumors and could be related to tumor progression and poor prognosis. Blood eosinophil count when massive tissue eosinophilia is present should be investigated upon.

A marked eosinophilic infiltrate is a microscopic feature that deserves proper attention and could be added to the histopathology report of oral cavity SCC along with other traditional macro- and microscopic parameters.

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**Conflicts of interest**
There are no conflicts of interest.

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