Quantitative analysis of tumor-associated tissue eosinophilia in different histological grades of oral squamous cell carcinoma

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ABSTRACT

Background and Objectives: Oral squamous cell carcinoma (OSCC) is the 8th most common aggressive epithelial malignant neoplasm worldwide today. The eosinophil may be a “multifaceted cell” that can be associated with wound-healing processes, as well as to tissue damage which has increased the speculations around tumor-associated tissue eosinophilia in malignant tumors. The aim of this study was to detect the role and quantitative analysis of tumor-associated tissue eosinophils in different histological grades of OSCC.

Materials and Methods: A retrospective study was carried out in sixty cases of histopathologically graded OSCCs. Tissue sections of 4 µ thickness were made from paraffin-embedded tissue blocks and were stained with hematoxylin and eosin. Eosinophils were counted under randomly selected twenty high-power (×40) fields. Data were subjected to statistical analysis using ANOVA test.

Results: Higher mean eosinophils were recorded in well-differentiated squamous cell carcinoma (WDSCC) followed by moderately differentiated squamous cell carcinoma (MDSCC) and poorly differentiated squamous cell carcinoma (PDSCC) groups, respectively. The difference in mean eosinophils was found to be statistically significant between WDSCC and MDSCC (P < 0.001), as well as between WDSCC and PDSCC (P < 0.001).

Conclusion: Tumor-associated tissue eosinophil count is higher in WDSCC as compared to moderate and PDSCC.

Key words: Grading, immunity, oral squamous cell carcinoma, tissue eosinophils

Oral squamous cell carcinoma (OSCC) is the 8th most common aggressive epithelial malignant neoplasm worldwide today.[1] Pindborg in 1997 defined squamous cell carcinoma as “A malignant epithelial neoplasm exhibiting squamous differentiation characterized by the formation of keratin and the presence of intercellular bridges.”[2] The vast majority of cancers in the oral cavity are squamous cell carcinomas. Its evolution is influenced by host immune response cells such as CD8+ T-cells, CD4+ T-cells, natural killer cells, dendritic cells, macrophages, and eosinophils.[3]

The eosinophil, which was discovered more than 120 years ago, is a “good looking” blood circulating granulocyte that is associated with numerous diseases and under normal conditions it is present at mucosal sites. Eosinophils are cells derived from the bone marrow characterized by the presence of specific granules containing cationic proteins such as the major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin, and the eosinophil peroxidase, which are strongly stained by eosin. Other inflammatory mediators are also synthesized and released by eosinophils, such as granulocyte-macrophage colony-stimulating factor,

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interleukin-3 (IL-3), IL-5, tumor necrosis factor-alpha, transforming growth factor-alpha (TGF-a), and TGF-b. Some of these factors are related to cell lysis including tumor cells.\(^4\)

The fact that eosinophil may be a “multifaceted cell” that can be associated with wound-healing processes, as well as to tissue damage has increased the speculations around tumor-associated tissue eosinophilia (TATE) in malignant tumors.\(^5\)

Thus, the aim of the study was to quantify and compare TATE in well-differentiated squamous cell carcinoma (WDSCC), moderately differentiated squamous cell carcinoma (MDSCC), and poorly differentiated squamous cell carcinoma (PDSCC) and assess the role of eosinophils in cancer.

**MATERIALS AND METHODS**

A retrospective study was carried out in sixty cases of OSCC, which were further divided into three groups, namely WDSCC, MDSCC, and PDSCC. Tissue sections of 4 micron thickness were made from paraffin-embedded tissue blocks and were stained with hematoxylin and eosin stain [Figures 1-3]. Eosinophils were counted under randomly selected twenty high-power (×40) fields (HPF). Quantitative analysis of TATE was done using image analysis (image proRes software, JENOPTIK optical system GmbH, Jena, Germany), and data were subjected to statistical analysis using ANOVA test.

**Inclusion criteria**

a. Histopathologically diagnosed cases of different grades of OSCC
b. Clinical data of the sample obtained from the records such as age, sex, habits, and site of the lesion.

**Exclusion criteria**

a. Patients with other simultaneous primary tumors
b. Metastatic tumors of oral cavity or jaw bones
c. Previous history of chemotherapy and radiotherapy.

**RESULTS**

In present study males seem to have marked predilection than females in all the three grades of OSCC [Table 1]. The mean, minimum & maximum age of distribution in different grades of OSCC were in the range of 55 to 59 years, 30 to 39 years and 72 to 75 years respectively [Table 2]. Overall age and number of cases distribution in SCC are shown in the Table 3.

Overall in OSCC, 4 patients (6.66%) were in the age group of 31-40 years, 15 patients (25%) were in the range of 41-50
years, 19 patients (31.66%) were in the range of 51-60 years, 17 patients (28.3%) were in the range of 61-70 years age group and 5 patients (8.33%) were above 70 years. Out of 60 patients of OSCC, buccal mucosa was the most common site followed by tongue, mandibular alveolar mucosa, maxillary alveolar mucosa, lower lip, floor of the mouth and retromolar region [Table 4].

Higher mean eosinophils were recorded in WDSCC group followed by MDSCC group and PDSCC group, respectively [Table 5]. The difference in mean eosinophils among the groups was found to be statistically significant (P < 0.001) [Table 6].

Multiple comparisons were done among different pair of groups using Bonferroni test of significance [Table 7]. The difference in mean eosinophils was found to be statistically significant between WDSCC and MDSCC (P < 0.001), as well as between WDSCC and PDSCC (P < 0.001). No significant difference was observed between MDSCC and PDSCC (P > 0.05).

### Table 1: Gender distribution in different histological grades of oral squamous cell carcinoma

| Group  | Male | Female | Total |
|--------|------|--------|-------|
| WDSCC  | 13   | 7      | 20    |
| MDSCC  | 13   | 7      | 20    |
| PDSCC  | 17   | 3      | 20    |

### Table 2: Mean age in different histological grades of oral squamous cell carcinoma

| Group  | Mean | Std dev | SE of mean | Median | Min | Max |
|--------|------|---------|------------|--------|-----|-----|
| WDSCC  | 55.10| 9.73    | 2.18       | 55.5   | 30  | 72  |
| MDSCC  | 54.40| 10.04   | 2.25       | 55.0   | 35  | 72  |
| PDSCC  | 58.90| 10.05   | 2.25       | 59.5   | 39  | 75  |
| Overall| 56.13| 9.97    | 1.29       | 57.0   | 30  | 75  |

### Table 3: Age distribution in the study group

| Age (years) | Number of cases | Percentage |
|-------------|-----------------|------------|
| 31-40       | 4               | 6.6%       |
| 41-50       | 15              | 25%        |
| 51-60       | 19              | 31.66%     |
| 61-70       | 17              | 28.3%      |
| 71-80       | 5               | 8.33%      |
| Total       | 60              | 100%       |

### Table 4: Site of the lesion

| Site                     | Number of cases | Percentage |
|--------------------------|-----------------|------------|
| Buccal mucosa            | 23              | 38.33      |
| Tongue                   | 16              | 26.66      |
| Mandibular alveolar mucosa| 10              | 16.66      |
| Maxillary alveolar mucosa| 8               | 13.33      |
| Lower lip                | 1               | 1.66       |
| Floor of the mouth       | 1               | 1.66       |
| Retromolar region        | 1               | 1.66       |
| Total                    | 60              | 100%       |

### DISCUSSION

Eosinophils are long-lived cells following their release from bone marrow. Usually multifunctional cells contain and produce many biologically active substances. Eosinophilia is seen in parasitic infections or allergic disorders. According to recent studies, eosinophil infiltration is also present in target tissues of both physiological and pathological processes, such as angiogenesis, embryogenesis, immune regulation, different infections, or neoplasia leading to tissue damage or remodeling. Parameters available in literature for intense TATE in head and neck tumors range from >10 up to 100 eosinophils per HPF.

The first case of malignant tumor with marked blood eosinophilia was described by Rheinbach in 1893; eosinophilia has been observed in many cases of carcinoma from various organs. There are only a few papers available, however, which have reported on the relationship of such eosinophilic infiltration or peripheral eosinophilia in the histologic findings of tumors.[6]

In the present study, a quantitative assessment of tissue eosinophils in diagnosed cases of OSCC was done. This has
enabled in the assessment of the role of these cells in the immune regulation of OSCC. In addition to that the incidence of OSCC in relation to age, gender, and habits was also noted.

In the present study, the overall age of SCC patients was 56 years. M Bryne confirmed that OSCC is a disease of the middle-aged and elderly with ages ranging from 40 to 75 years. It is also found in the present study that males have a marked predilection for OSCC in all three grades of carcinoma. This is in accordance with Shafer’s on OSCC which has shown that this entity has a striking male predominance.\(^2\)

The habits such as smoking and chewing tobacco with betel nut have been associated with an increased prevalence of OSCC.\(^3\) We found that all sixty patients (100%) included in this study had the habit of tobacco and betel nut chewing, and 37 patients (61.66%) had the habit of smoking. Probably these habits might have contributed to the development of carcinoma.

The distribution of cancer at various intraoral sites in different populations suggests the differences in risk factors. In the present study, 23 cases (38.33%) had lesions on the buccal mucosa and 16 cases (26.66%) had lesion on the tongue. Carcinoma of buccal mucosa and lateral tongue is frequently seen in betel quid chewers because the quid is placed against the buccal mucosa. In India, betel quid chewers constitute an important risk population and hence carcinoma of buccal mucosa and lateral tongue is most commonly seen in the Indian population.

Predominantly affected sites in smokers include retromolar area, floor of the mouth, lower lip alveolus, and tongue. The present study included one case (1.66%) each of carcinoma of lower lip, floor of the mouth, and retromolar region, respectively. Ten cases (16.66%) were of mandibular alveolar mucosa, whereas eight cases (13.33%) were of carcinoma of maxilla.

In the present study, the mean tissue eosinophil cell count (mean ± standard deviation) in WDSCC was 19.85 ± 9.583 cells/20 HPF. This was higher than that of the MDSCC, which showed 6.55 ± 2.78 cells/20 HPF. The PDSCC showed 4.6 ± 2.18 cells/20 HPF. This difference was found to be statistically very significant (\(P < 0.001\)). This can be substantiated by the study of Falconieri et al.\(^7\) who proposed that there are number of reports suggesting that high eosinophil densities that may be associated with tumor behavior in head and neck squamous cell carcinoma.\(^6\)

Higher mean eosinophils were recorded in WDSCC followed by MDSCC and PDSCC, respectively. The difference in mean eosinophils among the groups was found to be statistically significant (\(P < 0.001\)).

To our knowledge, there have been no earlier reports regarding the quantitative analysis of TATE in different histological grades of OSCC. The presence of only an increased number of eosinophils in the SCC has been reported earlier.\(^9\) This has been attributed to that the TATE was associated with significantly better clinical outcome for the patient with head and neck SCC.\(^10\)

A variety of stromal reactions are associated with invasive carcinomas. The most common responses are an infiltrate of inflammatory cells, especially mononuclear cells such as lymphocytes, plasma cells and desmoplastic proliferation of fibroblasts. Eosinophilic leukocytes are often seen among the inflammatory cells, but they seldom are the major constituent. About one-fourth to two-thirds of invasive carcinomas of the uterine cervix are accompanied by cellular reactions containing some eosinophils. Stromal reactions dominated by eosinophils have been reported in 4.4–13.65% of infiltrative cervical carcinomas.\(^11\)

Dorta et al.\(^6\) decided to perform an objective and reproducible evaluation of TATE in stage II and III OSCC by means of morphometric analysis. Their classification of TATE is based on the tertiles of the number of eosinophils per mm\(^2\) present in each of the 125 cases studied allowed them to compare discrete (0–26 eosinophils/mm\(^2\)) with intense (84 eosinophils/mm\(^2\)) degrees of TATE regarding prognostic influence. As a result, they demonstrated the influence of high-grade TATE toward a better prognosis of OSCC patients.

According to Falconieri et al.\(^7\) the importance of eosinophils is not well established, available data overall indicate that a rich eosinophilic stromal infiltrate is invariably associated with locally invasive carcinoma. Outside the head and neck, eosinophils have been especially observed in tumors of the female genital tract. Moreover, an increased count of eosinophils in tumor tissue was documented in patients who responded well to radiotherapy, whereas poor responders had fewer eosinophils.\(^7\)

Eosinophils can stimulate tumor angiogenesis through synthesis and release of potent angiogenic factors.\(^6\) Eosinophils also play a role in antibody dependent cell-mediated cytotoxicity and in the synthesis and release of cytokines that could contribute, either directly or indirectly to tumor toxicity. A number of reports have suggested that high eosinophil density may be associated with tumor behavior in head and neck squamous cell carcinoma.

Ishibashi et al.\(^10\) conducted a study on eosinophils in SCC and they showed that the number of TATE was significantly higher in cases without venous invasion, lymph node metastasis, and clinical recurrence. In particular, cases associated with a larger number of TATE infiltrations usually demonstrated better clinical outcomes than those which are not associated.\(^10\) These findings suggest the possible
correlation between TATE and less aggressive biological behavior of the tumor.

Bostrom et al.\textsuperscript{[11]} have shown the exact nature of the eosinophils has been enigmatic for decades. One of the prime roles of the eosinophil is the modulation of the mast cell and basophil response in immediate hypersensitivity reactions. A specific substance in mast cells has been isolated which is released when the cells are stimulated by antigen and have IgE bound to their cell membrane. This substance, eosinophil chemotactic factor of anaphylaxis, has a strong chemotactic effect on eosinophils by a mechanism as yet unknown.\textsuperscript{[11]}

Chatzistamou et al. showed chronic stromal inflammatory infiltration was significantly associated with tumor shape. Well-defined tumors were more prone to intense chronic inflammatory infiltration, which is associated with favorable prognosis.\textsuperscript{[12]} Human tumors associated with eosinophilia - leukemia, Hodgkin’s disease, B-cell lymphoma, T-cell lymphoma, plasma cell myeloma, mycosis fungoides, colon, stomach, pancreas, uterine cervix, lung, breast, ovary, endometrium, thyroid, and head and neck squamous cell carcinoma.\textsuperscript{[13]}

The presence of eosinophils within a wide variety of human cancers immediately raises two questions: Why are they present and what are they doing to the tumor? With regard to the first question, a number of factors have been shown to be potent eosinophil chemoattractants \textit{in vitro} and \textit{in vivo}, including platelet activating factor, C5a, RANTES, MCP-2, IL-5, eotaxin, and IgE. Of these factors, only IgE and IL-5 have to date been detected in lymphomas that are infiltrated by eosinophils. Unidentified eosinophil colony-stimulating factors, however, have been detected in the blood and tumors of patients with medullary carcinoma of the thyroid, melanoma, and adenocarcinoma of the biliary tree. It remains unclear if the tumor cells themselves or the host inflammatory cells are responsible for the production of these eosinophilotactic compounds.\textsuperscript{[13]} There is also clinical and laboratory evidence that a type 2 helper T-cell response and IL-4 may account for hypereosinophilia in some conditions, including tumors. This raises the intriguing possibility that the host immune response to the tumor results in the recruitment of eosinophils to the site.

Jacobsen et al. showed a new paradigm has emerged describing eosinophils as initial responders to cell death/tissue damage that are a part of remodeling/repair processes and more importantly, significant contributors to localized innate and acquired immune responses, as well as systemic adaptive immunity.\textsuperscript{[14]}

In the present study, the degree of TATE count is greater in WDSCC as compared to MDSCC and PDSCC. These findings are in agreement with Dorta \textit{et al.}\textsuperscript{[4]} and Lowe and Fletcher.\textsuperscript{[15]} They had noticed TATE has a favorable prognosis, suggesting that eosinophils may play an important role against epithelial tumors.

**CONCLUSION**

Tumor-associated tissue eosinophil count was higher in WDSCC as compared to MDSCC and PDSCC. Further analysis is required to confirm our results. In future, investigations related to the infiltration of eosinophils in the solid tumors are an interesting point to answer the important question: “Are eosinophils part of a host surveillance mechanism against the tumor?” The resolution of this question will open another route for new modalities of therapy of cancer patients, based on the activity of eosinophils.

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**Conflicts of interest**

There are no conflicts of interest.

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