

Tumor Associated Tissue Eosinophilia in Oral Squamous Cell Carcinoma: A Histo-Chemical Analysis

Priya SAHNI, Anil PATEL, Shylaja Md, Jayadeva HALLUR,
Pavan Kumar GUJJAR

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Department of Oral Pathology, Narsinbhai Patel Dental College and Hospital
Visnagar, Mahesana, Gujarat 384315, India

Abstract

Background: Tumor associated tissue eosinophilia (TATE) is believed to play a significant role in biological behavior of the carcinoma. Eosinophils are involved in immune reaction. Various studies have been carried out regarding their role in tumor progression or regulation. In oral squamous cell carcinoma (OSCC), eosinophils are associated with favourable or unfavourable prognosis and hence their role is yet unclear. To compare the tissue eosinophils in OSCC and normal tissue and to correlate the expression of TATE in different grades of OSCC.

Method: Study comprised 30 cases, 6 normal and 24 histopathologically diagnosed with OSCC. 4 micron thick sections were stained using 1% congo red solution. The sections were examined under high power ($\times 40$) and 10 consecutive microscopic fields were studied. The average number of eosinophils were statistically analysed.

Results: The tabulated results showed that the median value of tissue eosinophils, increased in OSCC compared to normal mucosa. Analysis on different grades of carcinoma showed a higher TATE in Well differentiated squamous cell carcinoma as compared to other grades.

Conclusion: The higher eosinophil count in OSCC compare to normal tissue might have a role in stromal invasion and infiltration. TATE can be used as an indicator of favourable prognosis in OSCC.

Keywords: Congo red stain, OSCC, TATE

Introduction

Oral cancer is the eleventh most common cancer globally. There is a wide geographical variation in the incidence of oral cancer, with approximately two-thirds of the patients being affected in the developing countries. In India oral cancer accounts for about 30% of all new cases annually and 22.9% of cancer-related deaths are due to oral cancer (1). The mortality and morbidity rates of oral squamous cell carcinoma (OSCCs) are very high (2).

TNM staging for OSCC is not always accurate in establishing a prognosis because its lack of ability to quantify biologic aggressiveness of tumor on cellular level. Similarly World Health Organization (WHO) grading alone shows poor correlation with outcomes and response to treatment in individual patient (1).

Tumor stroma consist of various inflammatory cells resulting from the host-response to tumor cell (2). Increased tissue eosinophil levels have been reported in various malignancies including OSCC.

In 1846, Wharton Jones described eosinophils as coarse granular cells (3); and later by Paul Ehrlich in 1880 as eosinophils (4). Eosinophils are pleiotropic, multifunctional leucocytes and play an important role in health and disease. Wide range of studies have demonstrated extensive tissue eosinophilia in OSCC (5).

Tumor-associated tissue eosinophilia (TATE) is defined as "eosinophilic stromal infiltration of a tumor, not associated with tumor necrosis or ulceration" (5). They are scattered both in and around the lesional cells, dividing them into minute groups (3). TATE was first described by Przewoskiin in 1896 in carcinoma of cervix, which was characterised by the presence of eosinophils as a component of peritumoral and intratumoral inflammatory infiltrate. TATE in malignancies is associated with different sites such as nasopharynx, larynx, esophagus, colon, cervix, oral cavity, so on (5).

Eosinophils are involved in immune reactions like inflammation and lysis of cells. Various studies have demonstrated their role in

tumor progression or regulation (6). They are hypothesized to have direct tumoricidal activity associated with release of cytotoxic proteins and act indirectly by enhancing the permeability of tumor cells; this allow the penetration of cytokines, which are responsible for the killing of tumor cells. They also produce several angiogenic factors for tumor progression through angiogenesis (5).

A highly potent and selective eosinophil chemoattractant, eotaxin - which mainly derived from tumor-associated eosinophils - is partly involved in eosinophils chemotaxis of the tumour. Likewise, mast cells secrete histamine and eosinophil chemoattractant factor (ECF), which further attract eosinophils in tissues (5).

Correlation of tissue eosinophilia with prognosis has shown variable result in OSCC. It has been related to favourable and unfavourable prognosis (5).

The aim of the present study was to elucidate the role of tissue eosinophilia in OSCC and predicting the prognostic value based on tissue eosinophils.

Materials and Methods

The study was carried out with sample size of 30 cases out of which 24 were OSCC and 6 were normal tissue. Tissue blocks were taken from archives of Department of Oral Pathology and Microbiology, Narsinhbhai Patel Dental College and Hospital, Visnagar. Normal tissue was taken from the patients undergoing tooth extraction for orthodontic treatment. The sample was distributed in three group after histopathological diagnosis: Group 1, well differentiated OSCC (WDSOC, n = 8); Group-2, moderately differentiated OSCC (MDSOC, n = 8); and Group-3, poorly differentiated OSCC (PDSOC, n = 8). Areas of tumor necrosis and degenerated muscle tissue were excluded from the study. For counting of eosinophils, 4 m thick sections were obtained and stained with 1% Congo red stain (5).

The sections were deparaffinised, hydrated through graded alcohol to water and then placed in 1% Congo red solution for 8 minutes followed by washing with water. Differentiation was carried out with 2.5% potassium hydroxide (KOH) solution. Then, the sections were counterstained with hematoxyline for 10 minutes. Differentiation was done with 1% acid alcohol. Lastly, the sections were dehydrated through alcohol and cleared in xylene and mounted with Diphenyl Xanthene (DPX). This resulted in eosinophils with red color granules.

Each section was viewed under high power (40x magnification) and ten consecutive microscopic fields were studied. Eosinophils per 10 high power fields (hpf) were noted (Figure 1) depicts the tissue eosinophils in normal tissue and different grades of OSCC.

Data was collected and subjected to statistical analysis using Man Whitney test and Kruskal Wallis non parametric test to compared eosinophils in grades of OSCC. This was followed by a post hoc Mann-Whitney test using SPSS software. A value of $P < 0.05$ was considered statistically significant.

Results

Table 1 shows the comparison of eosinophil infiltrate between normal tissue and OSCC. Median eosinophils per 10 hpf in normal tissue was 2 and in OSCC was 3. This showed a significantly increased TATE in OSCC ($P = 0.01$). The median number of eosinophils per 10 hpf in WDSOC, MDSOC and PDSOC was 5, 3 and 2 respectively. The comparison of TATE in different grades of OSCC showed higher TATE in WDSOC compared to MDSOC and PDSOC. The comparison between WDSOC and PDSOC showed highly significant result ($P = 0.006$); (Table 2).

Discussion

Globally, Oral cancer is an important cause of morbidity and mortality. The WHO is anticipating a greater incidence of OSCC in upcoming decades

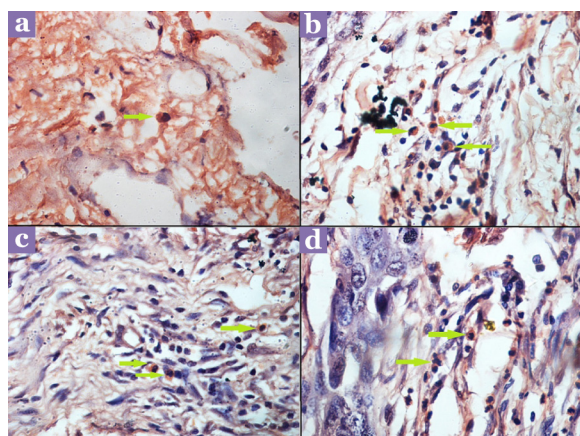


Figure 1: Congo red-stained normal tissue and different grades of OSCC sections showing tissue eosinophils (400x magnification). (a) Normal tissue, (b) WDSOC, (c) MDSOC, and (d) PDSOC.

Table 1: Comparison of TATE count in OSCC and normal tissue

| Group | Median | IQR | Non-parametric Mann-Whitney P value | Significance |
|-------------------------|--------|-----|-------------------------------------|--------------|
| Normal tissue | 2 | 1.2 | 0.01 | Significant |
| Squamous cell carcinoma | 3 | 0.8 | | |

Abbreviations: IQR = interquartile range; TATE = tumor associated tissue eosinophilia; OSCC = oral squamous cell carcinoma.

Table 2: Comparison of TATE count in different grades of OSCC

| Group | Median | Mean rank | P value | Significance | Post hoc Mann-Whitney test P Value | Significance |
|-------|--------|-----------|---------|--------------|------------------------------------|-----------------|
| WDSCC | 5 | 12.30 | 0.006 | significance | 0.08 | Not significant |
| MDSCC | 3 | 7.70 | | | | |
| MDSCC | 3 | 7.70 | | | 0.19 | Not significant |
| PDSCC | 2 | 4.00 | | | | |
| WDSCC | 5 | 12.30 | | | 0.006 | Significant |
| PDSCC | 2 | 4.00 | | | | |

Abbreviations: TATE = tumor associated tissue eosinophilia; OSCC = oral squamous cell carcinoma; WDSCC = Well differentiated squamous cell carcinoma; MDSCC = Moderately differentiated squamous cell carcinoma; PDSCC = Poorly differentiated squamous cell carcinoma.

around the world. Hence the search for factors with prognostic relevance is attracting attention to better monitor the individual management of OSCC patients (2).

Tumor invasion is considered a pathognomic feature of malignant tumor. Many host stromal factors, like endothelial and inflammatory cells, are also associated with tumor invasion along with genetic alteration. Immune cells scattered in the stroma of malignant tumors are considered a host immune response to the neoplasia (5).

Eosinophils are commonly encountered in human cancer, but their functional role in malignancy remains ambiguous. The presence of these cells in the tumor may be a result of eosinophilotactic substance released by tumor cells because of trapping of eosinophils in the stroma of the tumor (3).

The initial recruitment and activation of eosinophils towards the tumor microenvironment is a complex process which is mediated by inflammatory cytokines and chemokines and is principally related to the Th2 response. Interleukin-4 (IL-4) and IL-13 secreted by Th2 cells induce the production of potent chemokines, eotaxin which act as a chemoattractant for eosinophil (5).

TATE are hypothesised to have tumoricidal

activity involving the release of cytotoxic proteins and enhancement of tumor cells permeability, facilitating the penetration of tumor-killing cytokines. Tumor angiogenesis may be facilitated by the production of angiogenic factors. Pre-formed matrix metalloproteinases (MMPs) such as MMP-9 and tissue inhibitors of metalloproteinases-1 (TIMP-1) and TIMP-2 are released, which modulate extracellular matrix formation (5).

Therefore, the present study was carried out to elucidate the role of TATE in oral OSCC and its relevance as a prognostic indicator.

Comparison of median eosinophil infiltrates in the present study showed a significantly higher TATE in OSCC than normal tissue (Table 1). The different grades of OSCC when compared showed that WDSCC had a significantly higher TATE than the other grades ($P = 0.006$) (Table 2).

Infiltration of immunological cells in OSCC, as studied by Debta P et al., showed a mean value of TATE of 2.95 as compared to 1.90 in normal tissue. In accordance with this, the present study showed a higher median TATE in OSCC (7).

TATE, as elucidated in various studies such as those of Lowe and Fletcher (1984), Gold Smith et al. (1987), Gold Smith et al. (1992), Gao et al. (1997), Dorta et al. (2002), and Debta et al. (2011),

suggests that an increased number of tissue eosinophil is associated with antitumoural effects and a better prognosis (7).

TATE has also been studied in various other malignancies of the body like that of the larynx, oesophagus, nasopharynx, (8) which suggests its association with favourable prognosis; this is indicative of the good immune response of the body. It still remains unknown whether it is the eosinophils that lead to the improved prognosis or simply that the tissue eosinophilia is a coincidental epiphenomenon. Experimental evidences- has suggested that the growth of implanted tumours were inhibited if implanted site had eosinophilia. Direct damage to mammalian tumour cells by the eosinophil peroxidase system was also been demonstrated. Tumor necrosis factor (TNF) α , secreted by eosinophils is known to cause the death of tumour cells. All of these studies suggest that increased infiltration of tissue eosinophils is associated with the favourable prognosis and indicative of an anti-tumoural-role of TATE (2).

The studies by Wong DTW et al. (1990), Horiuchi et al. (1993), Van Driel et al. (1996), and Wong et al. (1999) in contrast suggest that tissue eosinophils play a tumor - promoting role in OSCC (7). Studies have demonstrated that patients with high eosinophil indices had a statistically significant lower survival than those with lower eosinophil indices (5).

Oliveira et al. (2009) found that TATE had no prognostic value in OSCC and suggested that intense TATE seems to reflect the stromal invasion of the OSCCs which occurs in advanced clinical stage (7).

The present study assessed the role of tissue eosinophila in OSCC. The increased tissue eosinophilia in WDSCC as compared to MDSCC and PDSCC indicated better prognosis. The higher tissue eosinophil count in OSCC compared to the normal tissue might have a role in the stromal invasion. TATE can be used as an indicator of favourable prognosis in OSCC.

Conclusion

TATE studied in OSCC and normal tissue showed an increased infiltration into the tumour stroma in which the well differentiated lesion had significantly higher infiltration than the lower grades. This could be an indicator that the eosinophils play a positive role to circumventing tumor invasion and its spread. The number and activity of the eosinophils need to be studied on large sample size to confirm the relevance of TATE for use as a prognostic indicator.

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Conflict of Interest

None.

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Correspondence

Dr Anil Patel
BDS (Gujarat University), MDS (III year Postgraduate)
(Hemachandracharya North Gujarat University)
Department of Oral Pathology
Narsinhbhai Patel Dental College and Hospital
SK campus, Kamana Cross Roads,
Visnagar – 384315
Gujarat, India
Tel: +91 903 353 0938
Email: anilpatel111120@gmail.com

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