

Assessment of role of eosinophils in the progression of tongue carcinoma



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ABSTRACT

Background: Tumor associated tissue eosinophilia (TATE) is known as stromal infiltration of eosinophils in the stroma of tumor not associated with tumor necrosis or ulceration. It has been described in a variety of neoplasms, but the role in oral squamous cell carcinoma (OSCC) of tongue has not yet been clearly defined. Association of TATE with OSCC has shown variable results ranging from favorable to unfavorable or even no influence on prognosis. **Aims and Objectives:** The aim of the present study was to correlate TATE with histopathological grades, lymph node status, tumor thickness and nature of tumor cells at the invasive front in OSCC of tongue. **Material and Methods:** Retrospectively fifty six cases of OSCC of tongue treated with radical neck dissection were included, which were divided into Group I consists 22 cases (lymph node with metastasis) & Group II consists 34 cases (lymph node without metastasis). H & E stained sections were used to analyze TATE and compared with histopathological grades, lymph node status, tumor thickness and nature of invasive front. Statistical analysis was done using Chi-square test. **Results:** TATE did not have any significant association with histopathological grades, lymph node status and nature of tumor cells at invasive front. But, tumor thickness and presence of eosinophils showed significant association. Also as the tumor thickness increased, the cells were more discohesive at the invasive front region. **Conclusion:** Microenvironment of tumor significantly contributes in the tumor progression. Tumor thickness, nature of tumor cells at the invasive front and TATE together can help in assessing the behavior of tongue squamous cell carcinoma.

Key words: Eosinophils, Invasive front, Metastasis, Tongue, Tumor thickness

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INTRODUCTION

Oral squamous cell carcinoma has a high possibility for local invasiveness and an increased tendency for cervical lymph node metastasis. Despite exercising clinical staging, histopathological grading and various treatment methods, the overall prognosis of patients has not improved significantly in the past 20 years, with 45-50% of 5-year survival rates. Prognostic value of tumor and stromal interactions has been neglected in the past.

Cancer associated stroma is a complex medium where a variety of interactions between tumor and stroma takes place. The stromal elements have prognostic as well as response-predictive information, and abundant

target therapy within the tumor microenvironment are being continually evaluated.¹ Tumor microenvironment is orchestrated by various components including the inflammatory cells, which consists of lymphocytes, plasma cells, neutrophils, macrophages, mast cells and eosinophils.² Most studies have mainly focused on lymphoplasmacytic infiltration; however limited literature is available on the role played by eosinophils in SCC of tongue specifically. Eosinophil infiltration in tumor microenvironment is known as tumor associated tissue eosinophilia (TATE).

Correlation of TATE with prognosis has shown variable results in OSCC. It has been related to a favorable as well as an unfavorable prognosis or even having no influence on patient outcome.¹⁻⁸ Therefore, the aim of this study was

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to determine the presence of TATE in SCC of tongue and to assess if a relationship exists between TATE and histopathological grades, lymph node metastasis, tumor thickness and nature of tumor cells at the invasive front.

MATERIALS AND METHODS

Fifty six paraffin embedded tissue blocks of histopathologically diagnosed cases of SCC of tongue based on Broder's system of grading, with concomitant neck dissections were retrieved from the archives of the Department of Oral and Maxillofacial Pathology, SDM College of Dental Sciences Dharwad and The Karnataka Cancer Therapy & Research Institute, Navanagar, Hubballi. The cases were categorized into 2 groups; Group I comprising of 22 cases positive for lymph node metastasis and Group II comprising of 34 cases negative for lymph node metastasis. Study sample consisted of 36 cases of Grade 1, 16 cases of Grade 2, 17 of Grade 3 and 1 case of Grade 4 OSCC. Tumor thickness was measured from the surface till the invasive front region; presence of eosinophils was studied at the invasive front region along with the nature of tumor cells at the invasive front. Chi-square tests were performed to analyze the data.

RESULTS

The incidence of tongue OSCC in young patients (<40yrs; 34%) is relatively low as compared to older age group (>40yrs, 66%). OSCC was common among males (80%) than females. The common risk factor noted among these patients was tobacco chewing (57%) followed by tobacco smoking (16%), combined habits (13%) and 14% did not have any history of habit. 79% of patients reported were in the clinically advanced stage III & IV at the time of diagnosis, where as 21% were at the clinical stage I & II (Table 1).

When histopathological parameters were considered, 86% of cases were well differentiated SCC and 14% comprised of moderate & poorly differentiated SCC (Table 2). Nature of invasive front studied showed that 77% of cases had discohesive or single cells and 23% had either pushing margin or cohesive cells (Table 3). Histopathologically, 39% of cases showed metastatic deposit in lymph node and 61% cases showed only reactive changes in lymph nodes (Table 4). The 77% of cases had tumor thickness greater than 4mm and 23% of cases less than 4mm (Table 5).

Among group I, 7 cases showed the presence of eosinophils and absence in 15 cases, whereas in group II, 15 cases had eosinophils and 19 cases did not reveal eosinophils. The presence or absence of eosinophils

Table 1: Details of distribution of cases according to age, habit and clinical stage of SCC of tongue

Parameters	Grouping	Number of cases (%)
Age	<40 yrs	19 (34)
	>40 yrs	37 (66)
Gender	Male	45 (80)
	Female	11 (20)
Habit	Tobacco chewing	32 (57)
	Tobacco smoking	9 (16)
	Combined	7 (13)
	No habit	8 (14)
Stage	I+II	12 (21)
	III+IV	44 (79)

Table 2: Correlation between tumor grading and eosinophils

Tumor grading of SCC of tongue	Eosinophils		Total (%)	p-value
	Absent	Present		
Well-differentiated	31	17	48 (86)	0.146
Moderately & poorly differentiated	3	5	8 (14)	
Total	34	22	56	

Table 3: Correlation between nature of tumor invasive front and eosinophils

Nature of tumor invasive front	Eosinophils		Total (%)	p-value
	Absent	Present		
1	10	3	13 (23)	0.172
2	24	19	43 (77)	
Total	34	22	56	

1- Cohesive cells in the form of islands, cords and strands, 2- Discohesive cells in the form of groups or single

Table 4: Correlation between lymph node metastasis and eosinophils

Lymph node metastasis	Eosinophils		Total (%)	p-value
	Absent	Present		
Group I	15	7	22 (39)	0.357
Group II	19	15	34 (61)	
Total	34	22	56	

Group I- Lymph node with metastatic deposit, Group II- Lymph node without metastatic deposit

among the histopathological grading and with or without metastasis to the lymph node did not show any statically significant difference.

Statistical significant difference was evident with tumor thickness and the presence of eosinophils. In the tumor thickness with less than 4 mm only 2 cases (2/13) showed the presence of eosinophils while 11 cases did not show the evidence of eosinophils. But as the tumor thickness increased (>4mm), 20 out of 43 cases showed the presence of eosinophils.

Table 5: Correlation between tumor thickness and eosinophils

Tumor thickness	Eosinophils		Total (%)	p-value
	Absent	Present		
<4 mm	11	2	13 (23)	0.044
>4 mm	23	20	43 (77)	
Total	34	22	56	

Table 6: Association between the tumor thickness and nature tumor cells at the invasive front region

Tumor thickness	Nature of invasive front		Total (%)	p-value
	1	2		
<4 mm	4	9	13 (23)	0.786
>4 mm	9	34	43 (77)	
Total	13	43	56	

Also, as the tumor thickness increased the cells were more discohesive at the invasive front region and statistically correlated with the eosinophils in the stroma (Table 6). Eosinophils and its relation with nature of tumor cells at the invasive front revealed 19 (19/43) cases showed presence of eosinophils in the presence of discohesive, isolated tumor cells. Whereas only 3cases (3/13) revealed the presence of eosinophils with the cohesive nature of tumor cells at the invasive front region.

DISCUSSION

Eosinophils function as key mediators in host defense primarily against various infections; its role in allergic diseases like asthma, atopic dermatitis, gastrointestinal disorders and hyper eosinophilic diseases is not destructive but mediates the pathology. Tissue eosinophils have been recognized in cancer for almost 120 years. Their role has been implicated in the carcinoma of esophagus, gastrointestinal tract, uterine, cervical, skin and oral cavity.⁹⁻¹³ Tumor associated tissue eosinophilia (TATE) was first described by Przewoski in 1896 in cervical carcinoma, characterized by presence of peritumoral and intratumoral eosinophil infiltrate. The evidence showing that eosinophils infiltrate into tumor sites suggests that they play a role in host tumor interactions.

The relationship between inflammation and cancer is more widely accepted; however, many of the molecular and cellular mechanisms mediating this relationship remain unresolved. Inflammatory cell infiltration around the tumor, especially at the sites of frank invasion, has been considered to play an important role in the biological behavior of tongue SCC.¹⁴ Most of the studies performed OSCC & TATE are from all different subsites. This could

be one of the reasons for the variation in the prognostic value ranging from good to poor to no prognostic correlation.

Emphasizing the fact that OSCC is a heterogeneous group of tumor, TATE should be analyzed based on the subsites. Tongue carcinoma is commonest and its behavior is different from the other sites. Also, there is an increased incidence of tongue CA in young people with over-representation of female. Recently, attention has been directed to the infiltration of TATE in tongue SCC. But the role of TATE, their prognostic value in tongue SCC still remains ambiguous.

Our study is an attempt to understand the role of TATE in tongue SCC at the invasive front. To the best of our knowledge, this is the first study to correlate TATE with histopathological grades, lymph node status, tumor thickness and nature of tumor cells at the invasive front in a large sample size.

Till date there is no universally accepted method for quantification and grading of TATE and this has led to conflicting results. Various methods used are mean values of TATE to grade them into low, moderate and heavy, density of TATE at the invasive front and rest of the tumor area, peritumoral and intratumoral areas, number of eosinophils per high power; different sample size and different method of eosinophils staining could result in variation in the result.^{3,7}

Eosinophils have protective role against epithelial tumor by releasing cytotoxic proteins like eosinophil peroxidase, major basic protein, eosinophil cationic protein, etc. and also by activating the immune system resulting in a better prognosis. Inhibiting the immune system, eosinophils play a role in promoting epithelial tumor growth, thus causing poor prognosis.¹⁵

Histopathological grading of tumor is done based on the differentiation of cells as well, moderate and poorly differentiated and is a standard method to assess the behavior of the tumor. In the present study, we did not get the significant correlation between TATE and histopathological grades of tongue SCC. Our findings are in accordance with a study in which no correlation was found between TATE and histopathological grading.^{8,16}

Alkhabuli and High did not find correlation between density of eosinophils and SCC differentiation. Similar, finding has been recorded by Tadbir et al. and Rahrotaban et al., but it was lower in poorly differentiated group.^{8,17} According to Jain M et al., non-metastatic group with well and moderately differentiated tumor had higher

mean eosinophilic count than metastatic group. In the metastatic group, well differentiated carcinoma had higher TATE count than moderately differentiated.² Iwaski et al. found that low degree of tumor cell differentiation was significantly associated with strong eosinophilic infiltration, thus suggesting that particular level of differentiation of carcinoma preferentially attract eosinophils into the site of lesion.¹⁰

Cervical lymph node metastasis is an important prognostic factor in OSCC. In studies by Jain M and Goldsmith et al., there was a significant increase in eosinophil counts in non-metastatic carcinoma than metastatic oral carcinoma.^{2,3} Falconieri et al. also confirmed the eosinophil-rich squamous cell carcinoma, although associated with metastatic involvement of cervical lymph node, showed less aggressive behavior as compared to ordinary SCC.⁴ Therefore, eosinophilia can be a favorable histopathological prognostic factor. But in the present study, in the presence of eosinophils, only 7 (7/22) cases showed the lymph node involvement and 15 (15/34) cases developed lymph node metastasis but did not show any statistical significant difference between the eosinophils with the lymph node involvement. Similarly, Alrawi et al. demonstrated that patients with high eosinophil indices had a statistically lower survival than those with lower eosinophil indices.¹⁸ Alkhabuli and High found significant correlation between eosinophil density and survival and lymph node metastasis.¹⁶ Oliveira D T et al. demonstrated the intense eosinophilic infiltration can be a significant feature to predict occult lymph node metastasis in early OSCC patients.¹⁹ Eosinophils at the primary or secondary metastatic site how do they migrate are explained by Lorena SC et al.,²⁰ The factors stimulating local migration of eosinophils are released by the tumor tissue i.e. chemotactic factors such as eotaxin has selective action on eosinophils - play a role in in-situ eosinophilic infiltration and maintenance.²⁰ Also, PGD2 derived and secreted by oral carcinoma play an important role via CRTH2 in eosinophil recruitment and subsequent anti-tumor activity through the action of eosinophilic cationic proteins.²¹

Ocular micrometer is used to measure tumor thickness by recording the measurements from the superficial granular layer of the tumor epithelium to the tumor cells at the deepest part of the invasive front. It was suggested that the tumor thickness greater than 5 mm of tumor depth showed probable cervical lymph node metastasis. The metastatic rate of 64.7% was found in the group of more than 5 mm of tumor depth. In contrast, only 5.9% cervical lymph node metastasis was observed with less than 5 mm of depth of invasion.²² This is supported by the fact that as the depth of tumor invasion increases, the presence of

lymphatic channels in the deeper connective tissue has a high propensity for cervical metastasis.

In the present study with tumor thickness greater than 4 mm, 19 cases (19/43) showed metastasis to the lymph node, tumor thickness with less than 4 mm, only 3 cases (3/13) showed metastasis. The role of eosinophils in the progression of tumor is by the release of chemical mediators. The presence of eosinophils was seen in 2 cases and 11 cases did not show the evidence of eosinophils with the tumor thickness less than 4 mm. But as the tumor thickness increased (>4 mm), 20 cases showed the presence of eosinophils and 23 cases did not show the evidence of eosinophils. The tumor thickness and eosinophils showed statistical significant difference.

Also as the tumor thickness increased, the cells were more discohesive at the invasive front region and statistically correlated with the eosinophils in the stroma. Invasive part of the tumor has their morphological and functional characteristics compared to rest of the tumor and dictates the biological aggressiveness of OSCC. Pattern of invasion at the invasive site is described as pushing, well delineated infiltrating borders to discohesive small groups.

There was significant correlation with eosinophils and nature of tumor cells at the invasive front. In situ hybridization study revealed eosinophils have the capacity to synthesize actively 92-kd gelatinase, which degrade the basement membrane and extracellular matrix which enhances the tumor invasion and increase in the tumor thickness.²³

CONCLUSION

Focus from epithelial changes has begun to change towards the microenvironment of the tumor, which significantly contribute in the tumor development. However, ambiguity still exists with respect to TATE and their exact role in prognosis of OSCC.

Tumor thickness, nature of tumor cells at the invasive front and TATE together can help in assessing the behavior of tongue squamous cell carcinoma. TATE can be considered as an important microscopic parameter during histopathological reporting.

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Authors Contribution:

KH - Concept and design of the study, reviewed the literature, manuscript preparation and critical revision of the manuscript; **SA** - Conceptualized study, literature search, statistically analyzed and interpreted, prepared first draft of manuscript and critical revision of the manuscript; **SC** - Concept of study, collected data and review of study; **DG** - Literature search, statistically analyzed and interpreted, prepared first draft of manuscript; **BRP** - Conceptualized study, literature search, statistically analyzed and interpreted.

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