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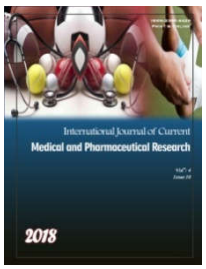

EVALUATION OF TISSUE EOSINOPHILS IN DIFFERENT GRADES OF ORAL SQUAMOUS CELL CARCINOMA USING H&E AND SIRUS RED STAIN

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ABSTRACT

Aim: To quantitatively evaluate and compare tumor associated tissue eosinophils in different grades of Oral Squamous cell Carcinoma by using H&E and Sirius Red Stain.
Materials and Methods: The study group consisted of totally 33 histopathologically diagnosed cases of Squamous Cell Carcinoma. Sections of 4µ thickness were made from each of the paraffin embedded tissues used in the study and were stained with H & E and Sirius Red Stain. Tumor associated tissue eosinophils (TATE) were counted using binocular light microscope equipped with square ocular grid.
Statistical Analysis:
To compare tumor associated tissue eosinophilia (TATE) among different histological grades of oral squamous cell carcinoma, one way ANOVA using SPSS software was used. To compare the specificity between Hematoxylin & Eosin and Sirius Red stain for the detection of TATE, Student's - T test was used. P values < 0.05 were considered statistically significant.
Results: Tumor associated tissue eosinophilia (TATE) values were counted and compared within different histological grades of oral squamous cell carcinoma and no statistically significant (P > 0.05) difference was found using both stains. However, a decreasing trend in average number of TATE per 10 high power fields (hpf) was found from well differentiated grade towards poorly differentiated grade. The specificity of Sirius Red for TATE detection was found to be highly significant (< 0.05%) as compare to H&E stain.
Conclusion: The results of decreasing levels of TATE with the advancing grade of SCC correspond to anti- tumor role of eosinophils, which still require further research to understand the mechanism of host response to malignancy so that TATE can be used as new immunological therapeutic measure for the cancer treatment.

INTRODUCTION

In 1863, Sir Rudolf Virchow observed leucocytes in neoplastic tissues and establish a connection between inflammation and cancer. Since then the inflammation-cancer connection has undergone a renaissance one of compelling new information from different areas of cancer research.[1] Tumor-associated tissue eosinophilia (TATE) is defined as "eosinophilic stromal infiltration of a tumor not associated with tumor necrosis or ulceration." [2] TATE has been found in squamous cell carcinomas of different locations, including the oral cavity.[3],[4] Some studies have reported favourable prognosis in tumors demonstrating tissue eosinophilia.[3] while others correlated intense TATE with poor prognosis. [5],[6] Thus over the years, the importance of tissue eosinophilic infiltrate has been reappraised from time to time, either as an important diagnostic tool for the assessment of stromal invasion or as a prognostic indicator. [7] Bearing in mind the prognostic

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significance of eosinophils in carcinomas, this study was designed to quantitatively evaluate and compare tumor associated tissue eosinophils (TATE) in different histologic grades of oral squamous cell carcinoma by using H&E and Sirius Red Stain.

MATERIAL AND METHODS

The study group consisted of totally 33 cases, including 15 Well Differentiated Squamous Cell Carcinoma, 12 Moderately Differentiated Squamous Cell Carcinoma and 6 Poorly Differentiated Squamous Cell Carcinoma. All the cases for OSCC were graded histopathologically by using Broders grading system. [8]

Two tissue sections of 4µ thickness were made from each of the paraffin embedded tissues. One section was stained with H & E, and the other with Sirius Red stain. Histopathological analysis of eosinophils was done using binocular light microscope equipped with square ocular grid. Eosinophils were counted under high power field (hpf) (10 ocular and ×40 objective lens) using an oculometer grid with 20 x 20 divisions. At invasive front region, eosinophils were counted in 10 successive grid fields by moving the section in a step ladder fashion. Results were recorded as eosinophils/10hpf. [9]

Only nucleated cells with intensely red cytoplasmic granules were accepted as eosinophils. Red blood cells with superimposed mononuclear and polymorphonuclear inflammatory cells were excluded (Figure 1& 2).

RESULTS

Tumor associated tissue eosinophilia (TATE) values were compared within different histological grades of oral squamous cell carcinoma and no statistically significant (P > 0.05) difference was found (Table-I). However, we found a decreasing trend in average number of TATE per 10 hpf's from well differentiated grade towards poorly differentiated grade.

Table I Comparison of TATE with different grades of Squamous Cell Carcinoma using Hematoxylin & Eosin and Sirius Red stain

Stains	Tumor differentiation	N	MEAN (TATE)	Std. Deviation	Sig
Hematoxylin and Eosin	WDSCC	15	28.447	22.2347	.107
	MDSCC	12	23.208	14.3018	
	PDSCC	06	9.717	5.8571	
Sirius Red	WDSCC	15	35.940	32.2499	.186
	MDSCC	12	28.675	16.8189	
	PDSCC	06	13.683	7.5669	

The mean difference is significant at the level of 0.05.

The specificity between H&E and Sirius red stain for the detection of TATE among different histological grades of oral squamous cell carcinoma was compared. A highly significant difference (< 0.05%) in between these stains for counting TATE was found. (Table II)

Table II Comparison between Hematoxylin & Eosin and Sirius Red stain for TATE count

Tumor differentiation	Stains	MEAN	N	Std. Deviation	Std. Error Mean	Sig (2-tailed)
WDSCC	Hematoxylin and Eosin	28.447	15	22.2347	5.7410	.016
	Sirius Red	35.940	15	32.2499	8.3269	
MDSCC	Hematoxylin and Eosin	23.208	12	14.3018	4.1286	.001
	Sirius Red	28.675	12	16.8189	4.8552	
PDSCC	Hematoxylin and Eosin	9.717	06	5.8571	2.3912	.010
	Sirius Red	13.683	06	7.5669	3.0892	

The mean difference is significant at the level of 0.05.

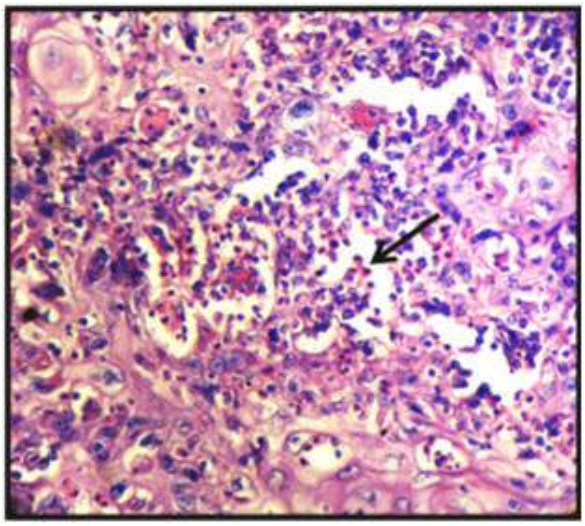

Figure 1 Eosinophils (arrow) in Oral SCC (H&E)

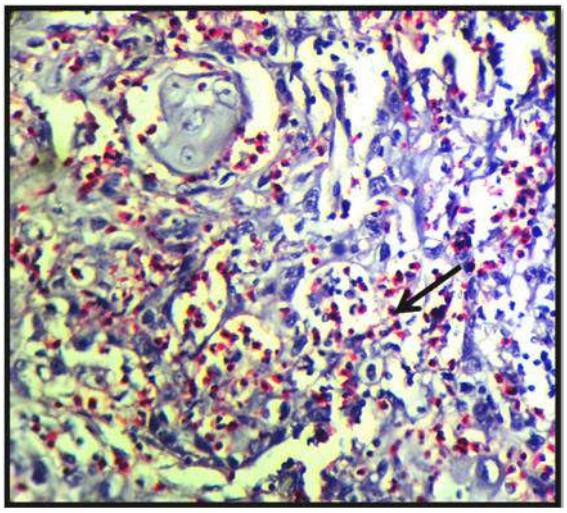

Figure 2 Eosinophils (arrow) in Oral SCC (Sirius Red)

DISCUSSION

Oral squamous cell carcinoma remains a serious oral health problem. Despite therapeutic and diagnostic progress in oral oncology, the prognosis of intraoral SCC remains poor.[10] Squamous cell carcinoma is an immunogenic tumor arising against a background of cellular immunodeficiency. The infiltration of human tumors by stromal cells is a subject of much curiosity and is often associated with a better prognosis, though this is not proven and is still controversial. [11]

Tumor associated tissue eosinophilia (TATE) in epithelial malignancies has been reported since 1896, still its role in carcinogenesis has remained unclear. Although literature demonstrates a tendency to consider TATE as a favorable prognostic factor in head and neck SCC, TATE has also been related to a poorer prognosis or even with no influence on patients' outcome, reflecting that this issue is still a matter of controversy. [12]

In the present study, the eosinophilic infiltrate was quantitatively evaluated. A variation in tumor eosinophilic infiltrate in 10 high power fields (HPF's) was found in different histological grades of squamous cell carcinoma. This can be attributed to individual variation in the host immune response to the tumor.

Decreasing trend in average number of TATE per 10 HPF, from well differentiated grade towards poorly differentiated

grade was found. (TABLES-I) Lowe and Fletcher suggested a possibility of a particular pattern of squamous differentiation and dedifferentiation to be linked with tissue eosinophil response. As the tumor gets progressively dedifferentiated, certain factors (cytokines, products of cell damage and necrosis) get released from the tumor cells and generate an exuberant response of the body to the tumor growth. [13]

This finding is consistent with Kiziltas¸ *et al*. who reported that that intensity of TE declined with increasing malignant potential of colonic epithelial neoplasms and may be used as diagnostic indicator. [14] Also Moezzi *et al*. found that the stromal eosinophilia was prominent in adenomas and it declines with progression through the adenomacarcinoma. [15] TATE has been known to have two non-overlapping activities: 1) a destructive effector function potentially limiting tumor growth as well as causing recruitment and activation of other leucocytes; and 2) immunoregulative and remodeling activities which suppress immune response and promote tumor proliferation. 16 The results with high TATE values in well differentiated squamous cell carcinoma can be explained as an attempt by the host tissue to resist and limit tumor growth. But as the tissue resistance was surpassed and the tumor started proliferating, the value of tissue eosinophils decreased as seen in moderately differentiated squamous cell carcinoma. In poorly differentiated squamous cell carcinoma, TATE values were further decreased, substantiating the immune modulating effect of eosinophils and thus explaining the proliferation of tumor by suppressing host immune response.

TATE values were then compared within different histologic grades of oral SCC and no significant ($P > 0.05$) difference was found. (TABLES-I) This is in accordance with the findings of Horiuchi K *et al*. They found that the degree of eosinophil infiltration was not statistically correlated with any clinical, histological and immunological factors, thus considering TATE to be an independent prognostic factor. [17] Similarly, Ercan I *et al* in their study found that the relationship between TATE and tumor differentiation, perineural invasion, vascular invasion, pathologic nodal stage and depth of invasion were not statistically significant.[18] Alkhabuli and High did not find any correlation between eosinophil density and SCC differentiation in cases of SCC of tongue.[19] Also, Rahrotaban *et al*. demonstrated no significant correlation between TATE and histopathologic grading, but it was lower in poorly differentiated group than in others in cases of HNSCC.[20]

In contrast to our study, Iwasaki *et al*. found a significant correlation between low degree of tumor cell differentiation and intense eosinophilic infiltration suggesting that some special histologic type of carcinoma may preferentially attract eosinophil into the lesion.[21]

Also we compared the specificity between H&E and Sirius red stain for the detection of TATE among different histological grades of oral squamous cell carcinoma. We found a highly significant difference ($< 0.05\%$) in between these stains for counting TATE. (Table II). This goes in accordance with D K. Meyerholz *et al* who found that Sirius Red method was preferred over H&E method for eosinophil detection. This method provides excellent tissue contrast for ease of eosinophil identification and cellular specificity, which are both useful for investigators screening large numbers of tissues.[22](Figure 2). Different authors have also used Sirius red for the detection of tumor eosinophils in the paraffin sections. [23, 24]

Thus from the above values it can be established that Sirius Red, which is a specific stain for eosinophils, is definitely a better stain than H & E for the detection of tissue eosinophils and also has a significant influence on the TATE values.

CONCLUSION

In conclusion, the present study assessed that tumor associated tissue eosinophilia (TATE) cannot be related to advancing tumor grade, thus may be used as an independent prognostic factor for oral squamous cell carcinoma. However, the decreasing levels of TATE with the advancing grade of SCC correspond to anti- tumor role of eosinophils which require futher studies to understand the mechanism of host response to malignancy so that TATE can be used as new immunological therapeutic modalities for the cancer treatment. Also, Sirius Red should be preferred over H&E for the detection of tissue eosinophils due to its better specificity which affects quantification of TATE values.

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