ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 13, Issue, 05, pp.17435-17438, May, 2021

DOI: https://doi.org/10.24941/ijcr.41455.05.2021

RESEARCH ARTICLE **OPEN ACCESS**

ASSESSMENT OF TUMOR ASSOCIATED TISSUE EOSINOPHIL (TATE) IN ORAL SQUAMOUS CELL CARCINOMA USING CARBOL CHROMOTROPE STAIN

***Nayannika Mongmaw, Dr. Deepak Bhargava and Dr. Puja Bansal**

School of Dental Sciences, Sharda University, India

ARTICLE INFO

Article History:
Received 19th February, 2021
Received in revised form
24th March, 2021
Accepted 25th April, 2021
Published online 28th May, 2021

Key Words

Tissue-Associated Tumor Eosinophil,
Oral Squamous Cell Carcinoma,
Carbol Chromotrope.

ABSTRACT

Eosinophils are known moderators of innate and adaptive immunity. However, studies focusing on the role of Tumor-Associated Tissue Eosinophils (TATE) in oral squamous cell carcinoma are limited, keeping which in mind, the present study was conducted. **AIM:** To evaluate tumor-associated tissue eosinophilia in mild and moderate oral squamous cell carcinoma. **METHOD:** 30 formalin-fixed paraffin-embedded blocks of histopathologically proven oral squamous cell carcinoma cases were retrieved from the department archive. Two sections of 4µm of each block were subjected to staining by routine Hematoxylin and Eosin stain and Carbol Chromotrope stain. TATE was graded based on the modified classical counting method (Alkhabuli and High, 2006). Statistical analysis was done using SPSS version 21, and $P<0.05$ was considered statistically significant. **RESULT:** Statistically significant association was found between TATE count and oral squamous cell carcinoma ($P<0.0001$). **CONCLUSION:** The positive correlation between TATE count and increasing oral squamous cell carcinoma grades could indicate its role as a prognostic marker. We further concluded that Carbol chromotrope couldbe a better stain for TATE than routine hematoxylin and eosin.

Citation: Nayannika Mongmaw, Dr. Deepak Bhargava and Dr. Puja Bansal. "Assessment of Tumor Associated Tissue Eosinophil (TATE) In Oral Squamous Cell Carcinoma Using Carbol Chromotrope Stain.*", 2021. International Journal of Current Research, 13, (05), 17435-17438.*

INTRODUCTION

Squamous cell carcinoma is defined as a malignant epithelial neoplasm exhibiting squamous differentiation as characterized by keratin and/or the presence of intercellular bridges.[4]According to GLOBOCON 2018 (IARC), oral cancer accounted for 10.4% of total number of cancer cases in India; being the most prevalent cancer in males (16.1%) and fourth most prevalent in females (4.8%). Oral squamous cell carcinoma accounts for 90-95% of oral cancer.[3] Eosinophils are a subpopulation of granulocytes. It was first described by Wharton Jones as "coarse granular cells" and later by Paul Ehrlich as "eosinophils". [1] Eosinophils are physiologically found in their phenotypic mature phase in the peripheral blood (<400/mm³), for a short span of approximately 18 h, followed by its migration into the gastrointestinal tract or thymus, where they reside under homeostatic conditions.[2] They are involved in the initiation and propagation of diverse inflammatory responses and modulators of innate and adaptive immunity. Tumor-associated tissue eosinophilia (TATE) is defined as "eosinophilic stromal infiltration of a tumor not associated with tumor necrosis or ulceration".[2] It is characterized by the presence of eosinophils as a constituent of peritumoral and intratumoral inflammatory infiltrate.[13] Intact eosinophils can usually be detected in tissue sections of tumors that are stained with hematoxylin and eosin. However, tissue eosinophils often assume an amoeboid or "medusa" cell configuration, especially in fibrous tissue, thereby making their recognition in routinely stained sections exceedingly difficult.[1] A few studies showed variable correlations between TATE and prognosis of Oral Squamous Cell Carcinoma (OSCC), with favorable and unfavorable results. Furthermore, eosinophils do not take prominent staining with routine hematoxylin and eosin, and thus, the use of special stains that can stain TATE more vividly is recommended. In view of this, the present study was done for the assessment of TATE and its possible role as a prognostic indicator in OSCC using Carbol Chromotrope, a special stain.

Corresponding author:** ***Nayannika Mongmaw,
School of Dental Sciences, Sharda University, India.

MATERIALS AND METHOD

It was a retrospective study and included 30 paraffin-embedded tissue blocks of oral squamous cell carcinoma which were retrieved from the departmental archive. Out of the 30 cases, 15 were of well-differentiated oral squamous cell carcinoma and 15 of moderately differentiated oral squamous cell carcinoma. The study was performed on 10% neutral buffered formalin fixed paraffin embedded tissue blocks. Two 4µm tissue sections were prepared from each paraffin-embedded blocks and subjected to staining with hematoxylin and eosin (H&E) and carbol chromotrope.

The H&E stained slides (FIGURE 1) were observed for the confirmation of diagnosis and carbol chromotrope stained slides (FIGURE 2) were graded for tumor associated tissue eosinophil (TATE). For TATE staining; the tissue sections were dewaxed and brought to water, followed by staining with Ehrlich hematoxylin for 10 minutesand bluing was done. The slides were then counterstained with carbol chromotrope solution at 37°C in an incubator for 30 minutes, washed, dehydrated, cleared and mounted with DPX. Total score of TATE per 10 hpf (40x) was calculated based on modified counting method by Alkhabuli and High (2006) (TABLE 1). Mean score was taken for each sample and subjected to statistical analysis by SPSS Version 21. $P < 0.05$ was considered to be statistically significant.

Table 1. Alkhabuli And High (2006) Scoring Of Eosinophils

EOSINOPHIL COUNT	SCORE
<50 cells	1
50-120 cells	2
>120 cells	3

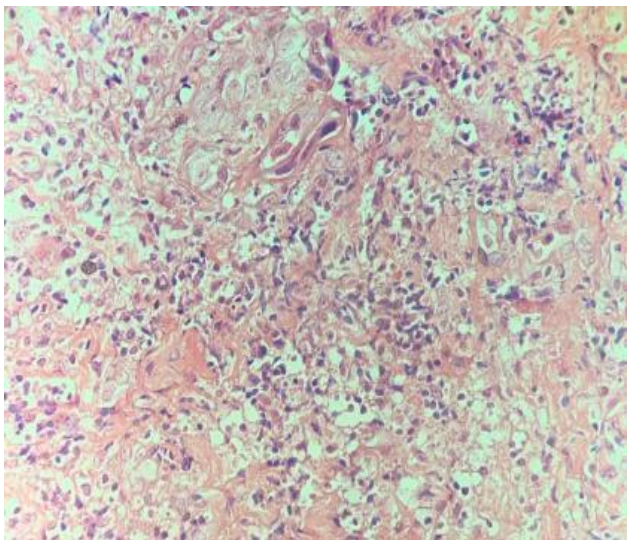


Figure 1. Hematoxylin & Eosin stain

RESULTS

Data was collected from all cases and tabulated in tables and graphs derived from statistical analysis for the interpretation of results.

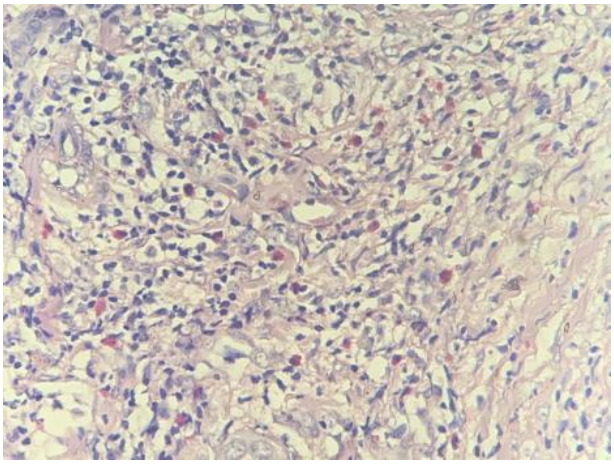


Figure 2. Carbol chromotrope stain (40x) shows presence of magenta coloured eosinophils in the tissue

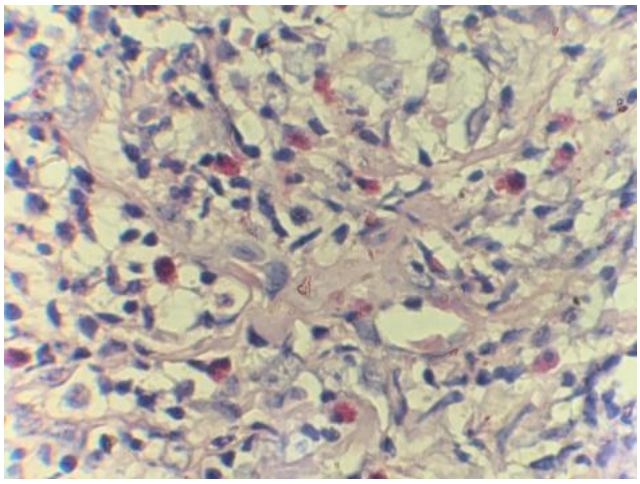


Figure 3. Carbol chromotrope stain (100x)

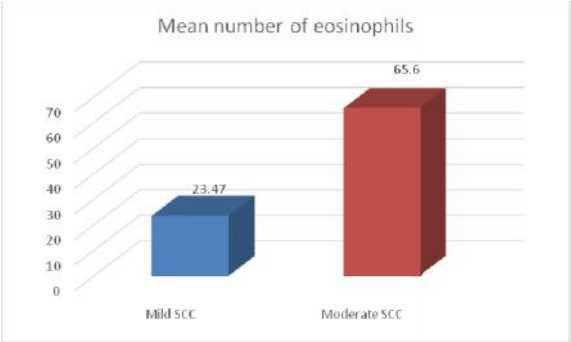
Among the 30 cases, mean number of eosinophils were 23.47 for well-differentiated OSCC and 65.60 for moderately differentiated OSCC which was statistically significant ($P<0.0001$) (TABLE 2 and GRAPH 1). On comparison of number of eosinophils in different grades of OSCC; 100% of well-differentiated OSCC showed score 1, whereas 33.3% of moderately differentiated OSCC showed score 1 and 66.7% showed score 2 (TABLE 3 and GRAPH 2).

Table 2. Intergroup Comparison Of Mean Number Of Eosinophils

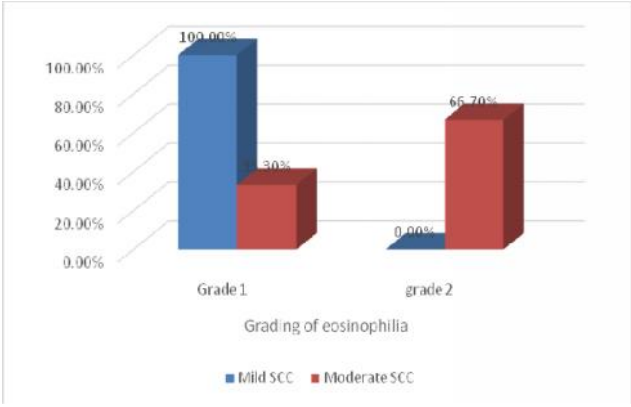
	SCC grading	N	Mean	Std. Deviation	P value
Eosinophil no	Mild	15	23.47	11.313	<0.0001, S
	Moderate	15	65.60	23.515	

Table 3. Intergroup comparison of grading of eosinophilia among mild & moderate oscc

			Grade of eosinophilia		Total
			1	2	
SCC grading	Mild	n	15	0	15
		%	100.0%	0.0%	100.0%
	Moderate	n	5	10	15
		%	33.3%	66.7%	100.0%
Total		n	20	10	30
		%	66.7%	33.3%	100.0%
P value			<0.0001, S		



Graph 1. Mean no of eosinophils was found to be significantly more among moderate scc as compared to mild scc.



Graph 2. Eosinophilia was found to be significantly more among moderate oscc cases as compared to that among mild oscc cases.

DISCUSSION

Eosinophils are bone marrow derived multifunctional granulocytes implicated in the pathogenesis of allergic reactions and parasitic infections.[4] They are distinguished by their tinctorial properties and abundant cytoplasmic granules.[1] Eosinophils can be identified in tissue sections stained with hematoxylin and eosin. Still, it can assume an unusual morphology, because of which the use of special stains such as carbol chromotrope and congo red is recommended.[13] Various studies have been conducted on tumor-associated tissue eosinophils in oral squamous cell carcinoma with conflicting results. In this study, eosinophils were found in almost all cases of oral squamous cell carcinoma. They showed an increase in the number of eosinophils with an increase in the grade of oral squamous cell carcinoma. This result is, according to another study by Majumdar B *et al.*; histopathological grading showed a positive correlation with the TATE count.[2] Tumors are complex tissues whose fate depends on the levels of pro versus anti-tumorigenic signals provided by the tumor cells by the local tumor microenvironment (including by resident and recruited immune cells) by the host systemically.[6] The eosinophils are thought to be recruited by the tumors in part by the highly potent and selective eosinophil chemo-attractant eotaxin, secreted by tumor-associated eosinophils, which recruits further cells from the bloodstream. Several other factors have been shown to be potent eosinophil chemo-attractants, in vitro and in vivo, including platelet-activating factor, C5a, RANTES, mcp-2, IL-5, and IGE.[5] Eosinophils have also been found to have a dual and divergent function, i.e., tumor promotive and tumor destructive. Eosinophils comprises of a primary and specific type of granules in their cytoplasm.[2]According to a study done by Davoine *et al.* in 2013; it suggests that eosinophils may contribute to the inflammatory response observed in OSCC and limit tumor progression by subsequent anti-tumor activity through the action of eosinophil cationic proteins.[7] The Eosinophil Cationic Protein is a ribonuclease, which partakes in various inflammatory diseases, and has immunomodulatory properties, and is cytotoxic to the epithelial cells.[10] The other major proteins of the specific granules include eosinophil peroxidase, major basic protein, and eosinophil derived neurotoxin. The major basic protein and eosinophil-derived neurotoxin have been found to have profound effects on dendritic cell maturation and functions.[11] Eosinophils respond to the signals produced by T-cells, as well as release preformed cytokines (interleukin-2, 4, 5, 10, 13; interferon-gamma) that promote either T1-helper or T2-helper cell responses. They can induce humoral cell responses such as priming B cells to manufacture antigen-specific IgM.[2] Pincus *et al.* demonstrated that eosinophil could stimulate fibroblast DNA synthesis.[8] Eosinophils are found to express transforming growth factor β1 (TGF-β1) and also contain preformed MMP-9 and inhibitors of MMPs, TIMP-1, and 2 having potent effects on the extracellular matrix modulation.[9] They can also act synergistically with macrophage reactive oxygen species and augment cytolysis of tumor cells or catalyze nitrite's oxidation to generate cytotoxic reactive nitrogen radicals.[10] According to a study by Jain M *et al.*, mean eosinophil count in the non-metastatic OSCC group was found to be significantly higher than metastatic group indicating that eosinophils have a good prognostic role in OSCC which is per Goldsmith *et al.* who found that TATE was significantly associated with favorable outcome in squamous cell carcinoma of head and neck.[13,14] Debta *et al.* found that increase infiltration of eosinophils and mast cells in OSCC were associated with favorable prognosis.[6] Another study conducted by Vaibhav SL *et al.*, eosinophilia increased from mild to intense with increasing grades of the OSCC cases. It was found intimately associated with ITF stroma and vascular components.[15] This finding is as per the present study. However, according to Joshi and Kaijkar, no correlation was noted between the eosinophilic infiltration and the histologic grades of OSCC.[16] According to Majumdar B *et al.*, there was mild to moderate eosinophilia in most OSCC cases, and tumor eosinophilia had a significant correlation with the pattern of invasion, suggesting its possible protective role in tumor cell cytotoxicity and progression.[2]

CONCLUSION

According to the present study, eosinophil infiltration directly correlates with the increase in grades of oral squamous cell carcinoma. However, there are various conflicting studies for the said matter. For further investigations, a standard universally recognized criteria for grading of tumor-associated eosinophilia is required. Special stains, such as carbol chromotrope and Congo red, are inexpensive and simplify eosinophils' detection, enabling good and rapid results. In the future, eosinophils may be used as an additional parameter in the grading of OSCC or as a prognostic indicator.

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