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TISSUE EOSINOPHILIA IN HEAD AND NECK SQUAMOUS NEOPLASIA: AN UPDATE

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Eosinophils are multifunctional granulocytes that play an imperative role in health and disease. They have also been found to be a crucial component of peri- and intratumoral inflammatory infiltrate. Tumor-associated tissue eosinophilia (TATE) has been observed and described in many tumors, including head and neck neoplasia. The process of eosinophil recruitment and its function in tumors has not been exactly defined yet. Correlation of tissue eosinophilia with prognosis has shown variable results ranging from favourable to unfavourable prognosis or even having no influence on patients outcome. Eosinophils are hypothesized to have tumor defensive as well as tumor promotive function. This dichotomous role of tissue eosinophilia with regard to prognosis has also been noted in head and neck neoplasia and premalignancies. So, the present review attempts to discuss TATE and its possible *pros* and *cons* in head and neck neoplasia.
Key Words: **eosinophils, tumor-associated tissue eosinophilia, head and neck squamous neoplasia, special stains.**

INTRODUCTION

Eosinophils are bone marrow derived multifunctional granulocytes implicated in pathogenesis of allergic reactions and parasitic infections [1, 2]. Tumor-associated tissue eosinophilia (TATE) was first described by Prezewoski in 1896 as "eosinophilic stromal infiltration of a tumor not associated with tumor necrosis or ulceration" [3]. TATE is characterized by presence of eosinophils as a component of peri- and intratumoral inflammatory infiltrate [4]. Literature search showed that TATE has been associated with malignancies of different sites such as oral cavity [4–12], nasopharynx [13], larynx [14, 15], esophagus [16], colon [17], cervix [18, 19], etc. Correlation of TATE with prognosis in malignancies of different sites including head and neck squamous cell carcinoma (HNSCC) have shown variable results ranging from favourable [4, 6, 13, 16] to unfavourable [12, 19] prognosis or even having no influence on patients outcome [3, 9]. Thus, the link between TATE and patients outcome is not exactly evident. Dualistic role of eosinophils might be attributed to the fact that eosinophils have direct and indirect tumoricidal activity as well as, they may promote tumor angiogenesis via production of several angiogenic factors [20]. The present review aims to discuss TATE and evaluate possible correlation between TATE and various clinicopathological parameters in head and neck squamous neoplasia through the data derived from available literature.

Detection of eosinophils in cancer

Eosinophils can be easily identified in tissue sections stained with hematoxylin and eosin (Fig. 1) but at times, these granulocytes assume an unusual morphology making their identification difficult particularly in fibrous tissue or inflammatory infiltrate. Such conditions entail the usage of special technique like autofluorescence, immunohistochemistry, luna staining and special stains like Congo red (Fig. 2) and carbol chromotrope for revelation of intact or degranulating eosinophils [21–24].

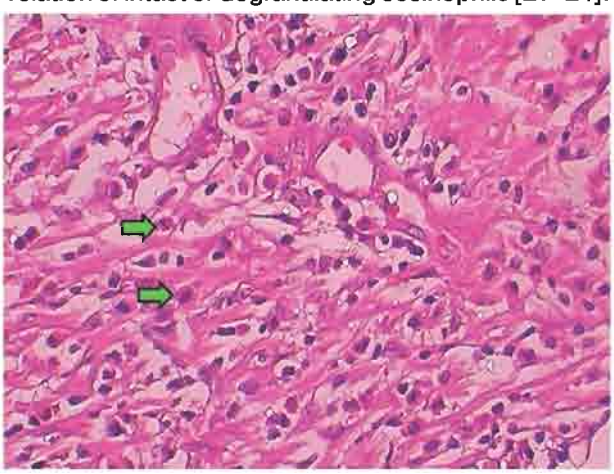

Fig. 1. Eosinophils in oral squamous cell carcinoma (OSCC) stained by hematoxylin – eosin, × 40

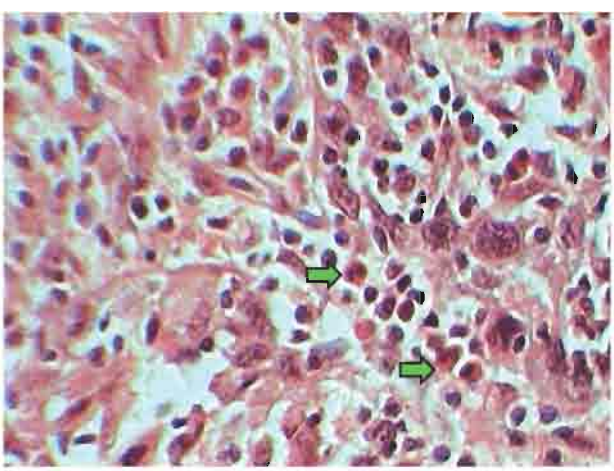

Fig. 2. Eosinophils in OSCC stained by Congo red, × 40

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Abbreviations used: DFS – disease free survival; HNSCC – head and neck squamous cell carcinoma; OSCC – oral squamous cell carcinoma; SPT – second primary tumor; TATE – tumor-associated tissue eosinophilia.

Criteria for identification of eosinophils

Literature reviewed suggested that only nucleated cells with intensely red cytoplasmic granules should be accepted as eosinophils. Care must be taken to exclude red blood cells with superimposed mononuclear and polymorphonuclear cells and also those confined to lymphovascular space must be disregarded [9, 15]. In addition, an effort should be made to discount eosinophilia associated with tumor necrosis or ulceration [3, 13].

Counting of tumor-associated tissue eosinophils

There are basically 2 methods described for eosinophil counting. In classical counting, eosinophils are counted per high power field (hpf) in sections of tumor or tumor edge or surrounding stroma randomly. In density method, the highest eosinophil density per surface area are counted using grid of definite dimensions [4, 25]. However, Alkhabuli and High [25] concluded that assessment of density may be better than classical counting and have more relationship with function.

Degree of TATE

Degree of TATE can be evaluated depending upon either counts only (number of eosinophils/mm^2 or hpf) as absent/mild, moderate, intense 4 or on the basis of both counts and distribution as focally and moderately elevated, focally and markedly increased, diffusely and moderately elevated, diffusely and markedly increased [15]. However, grading of TATE has got subjective variations since there is no consensus about the classification of TATE and different grading scales being used by various investigators [6, 8, 26].

Tissue eosinophils and HNSCC

TATE and clinical correlations in HNSCC

TATE and age and sex. Most of studies found that there is no correlation of TATE with age [4, 11] and sex [4, 5, 11, 13, 27] but, Ercan et al. [28] concluded that there is a negative correlation between TATE and age (in laryngeal squamous cell cancer) with lesser incidence of TATE over age of 60 years suggesting that age influences the tissue inflammatory response to tumor.

TATE and habits. Dorta et al. [4] and Oliveira et al. [5] did not found any association between TATE and tobacco and alcohol consumption in OSCC but Oliveira et al. [11] found that intense TATE is significantly associated with alcohol consumption only or with a long history of combined alcohol intake and cigarette smoking in OSCC.

TATE and tumor site. Dorta et al. [4] assessed TATE in OSCC of tongue, floor of the mouth, retromolar area and inferior gingiva and Oliveira et al. [11] analyzed TATE in OSCC of tongue and floor of the mouth but they did not found any statistically significant differences in the distribution of eosinophils among these sites.

TATE and TNM staging. Oliveira et al. [5] found that patients with OSCC in early stages T1/T2 presented absent/mild TATE, whereas intense tissue eosinophilia was strongly associated with tumors in advanced stages T3/T4. Dorta et al. [4] revealed that TATE is an independent prognostic factor and does not correlates with T and N clinical stage. Also, Oliveira et al. [11] and Thompson et al. [14] noticed statistically insignificant association between TATE status and tumor T category of patients with OSCC and SCC of larynx, respectively.

TATE and pathological correlations in HNSCC

TATE and stromal invasion. Said et al. [15] found frequently elevated (focally or diffusely) eosinophilic counts in invasive squamous neoplasia of larynx compared to preinvasive squamous neoplasia. Furthermore, increased eosinophil counts, specifically > 10/hpf and > 20/10 hpf were statistically significantly associated with stromal invasion. Thus, in an excisional specimen, if eosinophil counts meet these threshold, thorough evaluation of invasiveness is mandatory. Similar findings were reported by Alrawi et al. [8] in patients with *in situ* and invasive HNSCC. Our study [29] also found raised eosinophil counts in OSCC compared to oral epithelial dysplasia thus concluding that elevated eosinophil count is a histopathological marker associated with tumor invasion. Falconieri et al. [30] also concluded that eosinophil-rich SCC is constantly associated with stromal invasion and is of noteworthy especially in oral cavity cases where it becomes difficult to estimate due to initial small biopsy specimens. In such cases, marked tissue eosinophilia is suggestive of an SCC that is no longer confined within mucosal surface.

TATE and histological tumor type and tumor differentiation. Lowe and Fletcher [26] suggested that TATE development in skin and mucous membrane is in some way related to squamous differentiation of tumor since it is rarely reported in basal cell carcinomas and malignant melanoma. Also, it was proved that TATE is neither a site specific reaction nor a general disposition of patient to exhibit eosinophilia around any tumor. Moreover, they found that all of the tumors showing massive TATE were large cell poorly keratinizing, moderately differentiated and none was verrucous type SCC which possibly reflects that a particular pattern of squamous differentiation or dedifferentiation is linked in some way with tissue eosinophil response. Rahrotaban et al. [24] found that TATE was lower in poorly differentiated HNSCC but correlation between TATE and histopathological grading (Broders system) was not statistically significant. Also, Tadbir et al. [9] and Ercan et al. [28] did not found any significant relation between TATE and tumor differentiation in OSCC and laryngeal SCC, respectively. Looi et al. [13] observed TATE more frequently in non keratinizing nasopharyngeal carcinoma (NPC) compared to keratinizing SCCs and undifferentiated carcinomas.

TATE and its precise localization. Dorta et al. [4] found that eosinophil infiltrate location was statistically associated with TATE. In case of absent/mild/moderate TATE, eosinophils were exclusively limited to tumor stroma whereas in cases with intense TATE, eosinophils showed accumulation both in neoplastic clusters and tumor stroma. Another study [9] found mean eosinophil count/10 hpfs to be higher in invasive front of tumor than intratumoral stroma and stroma subjacent to epithelium but the correlation was not significant.

TATE and peripheral tissue infiltration/invasion. Few studies [5, 9, 11, 28] evaluated association of TATE with perineural, vascular, muscular glandular

and bone invasion but none of them found any significant correlation. Oliveira [5] observed eosinophilia in close association with damaged striated muscle fibers. Also, majority of OSCC with absent muscular infiltration showed absent/mild eosinophilia, although the correlation was statistically insignificant. So, they hypothesize that eosinophils might be involved in tissue remodeling through the release of their granules causing degradation of muscle fibers which are damaged by invasion of malignant cells.

Association of eosinophils with mast cells and inflammatory response. Debta et al. [10, 22] observed increased number of tissue eosinophils in OSCC cases accompanied by increase infiltration of mast cells. Increase infiltration of these cells were associated with improved prognosis indicative of antitumoral activity.

Dorta et al. [4] found statistically significant relation between mononuclear inflammatory infiltrate intensity and TATE in OSCC. This might involve a multifarious and cumulative interplay of many molecules including release of lymphocyte derived eosinophilotactic cytokines [4, 31]. Sassler et al. [27] observed an increased prevalence of TATE in those laryngeal SCC which demonstrated high degree of inflammatory response which might be attributed to tumor derived peptides causing chemotaxis of these cellular elements. Likewise, Lorena et al. [23] found intimate association of eosinophils with strong lymphoplasmacytic cell infiltration in OSCC. Looi [13] also noticed that eosinophils were present mainly between groups of neoplastic cells admixed with lymphocytes and plasma cells in NPC. On the contrary, Goldsmith et al. [6] did not found any correlation between grades of inflammation and eosinophilia in OSCC. Findings by Said et al. [15] also depicted that there is no association of elevated tissue eosinophils with overall inflammatory response denoting that elevated tissue eosinophilia is a specific cell response independent of a non-specific inflammatory reaction.

TATE and second primary tumor (SPTs)

Very few studies [5, 11] correlated TATE with SPTs but no statistically significant association was found.

TATE as a prognosticator in HNSCC

TATE and recurrence. Oliveira et al. [5] and Leighton et al. [3] concluded that TATE is not a significant predictor of locoregional and local recurrence in OSCC and NPC, respectively, where as Oliveira et al. [11] in OSCC noticed that TATE was highly correlated with regional recurrence but not with local recurrence. Study by Alrawi et al. [8] on HNSCC revealed potential association between higher eosinophilic index (EI >2) and locoregional recurrence suggesting that elevated eosinophilic counts is a predictor for aggressive tumor biology demanding close surveillance and additional therapeutic measures.

TATE and metastasis. Our study [29] found significantly increased eosinophil counts in non-metastatic OSCC compared to metastatic OSCC justifying TATE as a favourable prognosticator. Falconieri et al. [30] found heavy eosinophilia in all metastatic lymph nodes of eosinophil-rich OSCC indicating that tumoral rather than local factors are involved in eosinophilic migration. Moreover, eosinophili-rich SCC associated with metastatic involvement followed less violent course in contrast to ordinary SCC. Oliveira et al. [11] suggested that TATE is a significant marker to predict occult lymph node metastasis in patients with early OSCC and also an adjunctive histopathological factor to emphasize the call for elective neck dissection of patients with clinically N0 early OSCC. Goldsmith et al. [32] found that high grade TATE was statistically associated with absence of distant metastasis but the underlying pathogenetic mechanism remains unknown. Looi [13] observed that in NPC, TATE in the primary tumor was not always associated with eosinophilia in the metastases. Leighton et al. [3] concluded that TATE is not a significant marker of distant metastasis in NPC. Tadbir et al. [9] and Ercan et al. [28] did not found any significant correlation between TATE and locoregional metastasis in OSCC and SCC of larynx, respectively.

TATE and treatment. Oliveira et al. [5, 11] did not noted any significant association between intensity of TATE and radiotherapy in OSCC. Sassler et al. [27] found that presence of TATE could not be used to predict response to induction chemotherapy.

TATE and survival. Dorta et al. [4] found that in OSCC, 5 year disease free cumulative survival and overall cumulative survival was better in patients with intense TATE (72% and 63%, respectively) compared to those with absent/mild (32% and 27%, respectively) and moderate (44% and 37%, respectively) TATE. Thompson et al. [14] also demonstrated that over 5 years, TATE positive patients with laryngeal SCC have significantly improved survival compared with TATE negative patients. Moreover 1 year disease free survival (DFS) for TATE positive patients was 84% compared with 67% for TATE negative patients. Debta et al. [10] in OSCC cases found that patients who had survived for 3 years or more have raised tissue eosinophil counts in contrast to those who had survived for less than 3 years. So, above mentioned findings suggests that patients with higher numbers of tumor associated tissue eosinophils had a better prognosis than patients with intermediate or low counts. On the contrary, study by Alrawi et al. [8] in head and neck neoplasia cases demonstrated that patients with high EIs (3 and 4) had a statistically significant lower survival than those with lower EIs (1 and 2). In addition, patients with higher EIs had a significantly worse disease specific survival. However, Oliviera et al. [5, 11] observed that there is no differences in the 5 year and 10 year overall survival and DFS rates between the OSCC patients with absent/mild and intense eosinophilia and thus tissue eosinophilia is not a significant risk factor for death in OSCC patients. Similarly, Leighton et al. [3] in NPC, Ercan et al. [28] and Sassler et al. [27] in laryngeal SCC concluded that TATE is not a significant predictor of survival. Hence, TATE has got variable prognostic role in head and neck neoplasia.

DISCUSSION

Cancer induced inflammatory response, further termed as "immune surveillance" by F.M. Burnet

involves host defensive response against tumors via array of infiltrating lymphoid and myeloid cells which might either promote or retard tumor progression [33, 34]. Leucocyte infiltrate often fluctuate with type and size of tumor implying that immune responses are neither consistent nor static events [35, 36].

Eosinophils found to have dual and divergent function, i.e., tumor promotive as well as tumor destructive. Role of eosinophils in remodeling of connective tissue and formation of collagen was first proposed by Bassett in 1962 [21, 37]. Pincus et al. demonstrated that eosinophil can stimulate fibroblast DNA synthesis [38]. 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 [39, 40]. Eosinophils can amend the tissue remodeling process through the release of their distinct granule proteins. Eosinophil cationic protein inhibits proteoglycan degradation and promotes intracellular glycosaminoglycan accumulation in fibroblasts [41]. Major basic protein and eosinophil derived neurotoxin exhibit profibrogenic features stimulating fibroblast proliferation [42]. Eosinophils infiltrating tumors also known to release angiogenesis promoting factors like transforming growth factor-α (TGF-α) [43], vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (b-FGF) [44]. On the contrary, eosinophils can exhibit antitumor response via direct and/or indirect mechanism. *In vitro* studies have shown that eosinophils might cause direct cytotoxicity to tumors through release of their granules [45]. Indirectly, eosinophil peroxidase can act synergistically with macrophage reactive oxygen species and augment cytolysis of tumor cells [46] or catalyse the oxidation of nitrite to generate cytotoxic reactive nitrogen radicals [47].

Eosinophils can be related to cytokines of Th1 and Th2 response via synthesis and release of IFN-γ and IL-4, IL-5, and IL-10 [48, 49]. In HNSCC, it has been shown that the Th1 response is mainly associated with a better prognosis than those with the Th2 response [50, 51]. Early stage of OSCC was found to express mainly INF-γ and IL-2 genes (Th1 responses) whereas the advanced stage tumors have IL-4 and IL-10 expression (Th2 response) [52].

Eosinophils have been recognized and reported as inflammatory cellular infiltrate of various human cancer particularly of epithelial origin including OSCC [4–19]. However, the precise role of eosinophils in tumor growth and patient survival is still doubtful. Eosinophil recruitment in these tumors might be allied to release of several factors which have been shown to be effective eosinophil attractant in *in vitro* and *in vivo* studies [21]. So, in nutshell, with regards to above data and available literature, although eosinophil recruitment in head and neck neoplasia has been supported by various facts and evidences but the functional role of eosinophils in human cancer remains obscure i.e, are eosinophils restraining tumor growth as a part of host surveillance mechanism or do they augment tumor growth by remodeling and immunoregulation of tumor microenvironment or even have no effect on tumor evolution? Studies related to prognostic value of TATE in HNSCC has emerged with conflicting results which demands further investigations elucidating precise function of eosinophils as a prognosticator in head and neck neoplasia.

CONCLUSION

Present review is an effort to converse about different facet of TATE and its possible role and upshots in various head and neck squamous neoplasia. To our knowledge very little literature is available regarding same hence we hope that this review will contribute and add to present literature. Referred articles illustrated that TATE is an adjunctive microscopic feature that has momentous contribution to various clinico-histopathological parameters in HNSCC including the patient outcome. So, it is recommended that quantitative assessment of eosinophils should become part of the routine diagnosis. Exact mechanism of these cells whether they are involved in tumor inhibition or tumor promotion is still unknown and warrants further researches.

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