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Research Article

A HISTOMORPHOMETRIC STUDY OF EOSINOPHILS AND MAST CELLS IN POTENTIALLY MALIGNANT DISORDERS AND ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Background: Cell-to-cell interactions of mast cells and eosinophils are important for defense against the allergen in various infectious diseases, stromal invasion and carcinoma process. These cells have been shown to play an important role in progression of the oral potentially malignant disorders into squamous cell carcinoma. Therefore, a histomorphometric study of eosinophils and mast cells in potentially malignant disorders and oral squamous cell carcinomausing congo red and Toluidine blue stains, respectively was carried out.

Materials and Method: 10 cases each of histopathologically diagnosed mild dysplasia, moderate dysplasia, severe dysplasia, well-differentiated squamous cell carcinoma, moderately-differentiated squamous cell carcinoma and poorly-differentiated squamous cell carcinoma were selected. Each section was viewed under high power in 10 consecutive microscopic fields for counting of eosinophils and mast cells. Comparison of eosinophil and mast cell counts among different grades of dysplasia and stages of squamous cell carcinoma were assessed by both parametric one way ANOVA and non-parametric Kolmogorov-Smirnov test.

Result: It was found that the eosinophil counts showed an increasingly significant correlation in progression of dysplasias while mast cells did not show any correlation with such progression. While at the invasive fronts in oral carcinomas, mean mast cell counts showed a significant correlation while no such correlation is observed on observing eosinophils.

Conclusion: Thus, the present study concluded that, with progressive dysplasias, there was a correlating increase in eosinophils but not that of mast cells whereas in carcinoma sections, there appeared to be a correlating increasing mast cell counts with progression of the disease but not that of eosinophils.

INTRODUCTION

Potentially malignant disorders are the most common malignant lesions of the oral cavity which, may involve any intraoral site.[1] The classic histopathological alterations observed in oral potentially malignant lesions are cytological and architectural changes based upon which, oral potentially malignant disorders can be graded into mild, moderate and severe epithelial dysplasia.[2]. It has been established that oral potentially malignant disorders are more likely to progress to oral squamous cell carcinoma, if stimulus persist. Histopathologically, squamous cell carcinoma is classified into well differentiated squamous cell carcinoma, moderately differentiated sqauamous cell carcinoma and poorly differentiated squamous cell carcinoma.[1] It has been reported that mast cells and eosinophils co-exist in tissues, wherein IgE/antigen induced mast cells activation leads to the release of pro-inflammatory mediators which in turn, induce the recruitment of inflammatory cells, i.e., macrophages, T cells, eosinophils and basophils.[3]

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These cells, particularly eosinophils and mast cells have been cited in literature to aid in disease progression initiating from oral potentially malignant lesions to carcinoma development. However, there are studies lacking the concomitant expressions of these cells in same studied tissues that can aid in identifying their distribution and suggest a role in this spectrum of disease development and progression.

Although, eosinophils and mast cells can be easily observed in tissue sections when stained with hematoxylin and eosin staining, but sometimes these granulocytes are difficult to recognize. In such situations, special stains like Congo red and Toluidine blue prove to be a valuable diagnostic tool for detection of eosinophils and mast cells, respectively. Thus, the present study is aimed to elucidate the density of mast cells and eosinophils in oral potentially malignant disorders and oral squamous cell carcinoma using the two stains Toluidine blue and congo red respectively.

MATERIALS AND METHOD

Study was approved by the institutional review board. As per ethical guidelines prescribed by Declaration of Helsinki, all patient-related information was kept confidential and was not revealed in any form. 10 cases each of histopathologically diagnosed mild dysplasia, moderate dysplasia, severe dysplasia, well-differentiated squamous cell carcinoma, moderately-differentiated squamous cell carcinoma and poorly-differentiated squamous cell carcinoma of buccal mucosa were selected. Special staining of above mentioned cases using Toluidine blue and Congo red was performed for parallel sections of 4 micrometer thickness by using semiautomatic microtome **(**Shandon Finese E/ME**)** on albumin coated slides for Toluidine blue and Congo red stainings. The sections were stained using the following staining techniques:

1. Standard Harris's haematoxylin and eosin staining.
2. 1% Toluidine blue staining for mast cells.
3. 1% Congo red staining for eosinophils.

Hematoxylin and eosin staining was done for confirmation of the diagnosis of the lesions and to confirm the presence of satisfactory tissue.

Procedure for Toluidine Blue Staining For Mast Cells

Tissue sections were dewaxed in two changes of Xylene for 5 minutes each followed by hydration of the sections through descending grades of ethyl alcohol (100%, 90%, 75%, and 50%). Sections were then washed in running tap water for 5 minutes. They were then dipped in 1% Toluidine blue solution at pH 4.0 for 1 minute. The stained sections were then washed well in running water and followed by air drying. The stained sections were mounted with DPX (dibutylphthalate xylene) and cover-slippped.[4]

Procedure for Congo red Staining For Eosinophils

Tissue sections were dewaxed in two changes of Xylene for 5 minutes each followed by hydration of sections through descending grades of ethyl alcohol (100%, 90%, 75%, and 50%). Sections were then washed in running tap water for 5 minutes. They were then dipped 1 % Congo red solution for 5 minutes. The stained sections were then washed well in running water and followed by a dip in 2.5% KOH solution. Stained section were then counterstained with Harris haematoxylin followed by washing in running tap water. They were then dehydrated through ascending grades of alcohol (70% - 5 min, 90% - 5 min and absolute alcohol - 5 min) and cleared through two changes of Xylene for 5 minutes each. This was followed by mounting with DPX and coverslipping.[5]

Evaluation of Mast Cells and Eosinophils: The freshly stained tissue sections were immediately observed at 40x using binocular light microscope (Nikon research microscope ECLIPSE 80i). For determination of mast cell and eosinophil counts, counting of cells was performed at two different levels described as zone I and zone II. Maximum of 10 hotspots (positive fields) were selected.

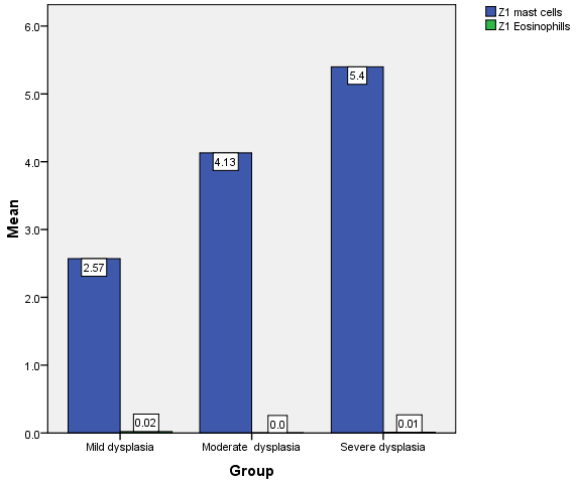

Figure 1 Bar diagram showing mast cell and eosinophilsin zone I in mild dysplasia, moderate dysplasia and severe dysplasia.

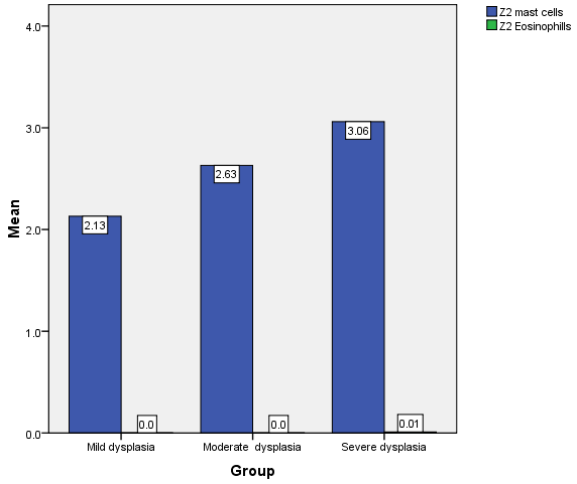

Figure 2 Bar diagram showing mast cells and eosinophilsin zone II in mild dysplasia, moderate dysplasia and severe dysplasia.

Table 1 Values for mast cells and eosinophils in mild dysplasia, moderate dysplasia and severe dysplasia in zone I and zone II.

Histological Grades Of Dysplasias	MAST CELLS		EOSINOPHILS	
	Zone I	Zone II	Zone I	Zone II
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Mild dysplasia	2.57 ± 1.82	2.13 ± 2.57	0.02 ± 0.04	0.00 ± 0.00
Moderate dysplasia	4.13 ± 1.09	2.63 ± 0.94	0.00 ± 0.00	0.00 ± 0.00
Severe dysplasia	5.40 ± 4.89	3.06 ± 2.74	0.10 ± 0.03	0.01 ± 0.03

The two zones were described as follows: ZONE I-Sub-epithelially within the inflammatory cell infiltrate. ZONE II-Within the deeper connective tissue layer.

For oral squamous cell carcinoma, mast cells and eosinophils were counted at the invasive fronts. Here also, a maximum of 10 hotspots (positive fields) were selected.

Statistical Anaylsis

Mean ± SD values for mast cells and eosinophils in mild dysplasia, moderate dysplasia and severe dysplasia in zone 1 and zone II (figure 1 & 2 respectively) was observed as tabulated in table 1 and in well differentiated, moderately differentiated and poorly differentiated SCC in table 2for mast cells and eosinophils (figure 3 & 4 respectively) by using Kolmogorov-Smimov Test.

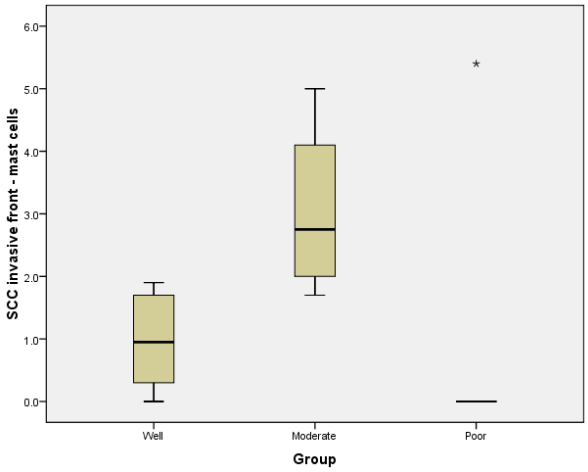

Figure 3 Bar diagram showing mast cells at invasive front in well differentiated carcinoma, moderately differentiated carcinoma and poorly differentiated carcinoma.

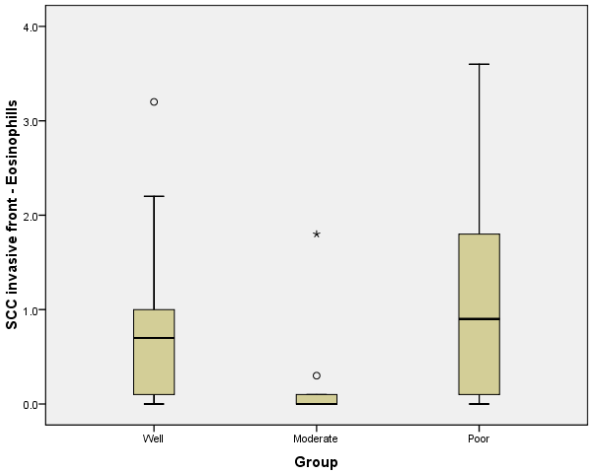

Figure 4 Bar diagram showing eosinophils at invasive front in well differentiated carcinoma, moderately differentiated carcinoma and poorly differentiated carcinoma.

RESULTS

It was observed that in mild dysplasia, moderate dysplasia and severe dysplasia eosinophil counts were (P = 0.00) statistically highly significant in all the histological grades of dysplasia but not for mast cells (Table 3).

Table 2 Values for mast cells and eosinophils in well differentiated, moderately differentiated and poorly differentiated SCC at invasive front.

	Mast cells	Eosinophils
Well differentiated SCC	0.97 ± 0.74	0.91 ± 1.04
Moderate differentiated SCC	3.10 ± 1.11	0.23 ± 0.55
Poorly differentiated SCC	1.08 ± 2.41	1.28 ± 0.98

Kolmogorov-Smirnov test, mean ± SD

Table 3 P value for mast cells and eosinophils in mild dysplasia, moderate dysplasia and severe dysplasia in zone I and zone II.

	Mast cells		Eosinophils	
Histological Grades of Dysplasias	Zone I	Zone II	Zone I	Zone II
Mild dysplasia	0.59	0.19	0.02	0.00
Moderate dysplasia	0.39	0.57	0.00	0.00
Severe dysplasia	0.43	0.31	0.00	0.00

It was also observed that P value at invasive front for histological grades of SCC was not significant for both mast cells as well as for eosinophils (Table 4). On applying ANOVA test, significant correlation was observed for mean number of mast cells at the invasive front in three carcinoma grades (P = 0.00, statistically highly significant) while mean eosinophil count showed no correlation (P = 0.13, statistically not significant).

Table 4 P values for mast cells and eosinophils in well differentiated, moderately differentiated and poorly differentiated SCC at invasive front.

Histological Grades of SCC	Mast cells	Eosinophils
Well differentiated SCC	0.98	0.48
Moderate differentiated SCC	0.79	0.09
Poorly differentiated SCC	0.21	0.98

DISCUSSION

Mast cells are bone marrow–derived cells, extensively distributed in the tissues.[6] When a mast cell, bind with IgE antibodies on exposure to the specific allergen, it result in a series of reactions which eventually leads to the release of series of powerful mediators which are responsible for setting into motion the events that lead to the late-phase reactions.[7]Among the mediators, Leukotriene $B_4$and platelet-activating factor are highly chemotactic for eosinophils, and monocytes.[8] At higher concentrations, it activates the inflammatory cells, causing them to degranulate.[9] Thus, these recruited cells found to be a crucial component of peri- and intratumoral inflammatory infiltrate in histolgical grades of dysplasia and SCC.[10] Although it is established that oral potentially malignant lesions and epithelial dysplasia are statistically more likely to progress to cancer.[1] At present, there are no molecular markers which enable us to distinguish lesions that may progress from those that will not.[11] At present, therefore the degree of dysplasia is the best guide to potential progression of oral lesions.[1] In this study, histopathological picture showing mast cells and eosinophils interaction in dysplasia and stromal invasion during carcinoma process at 40x magnification (figure 5).

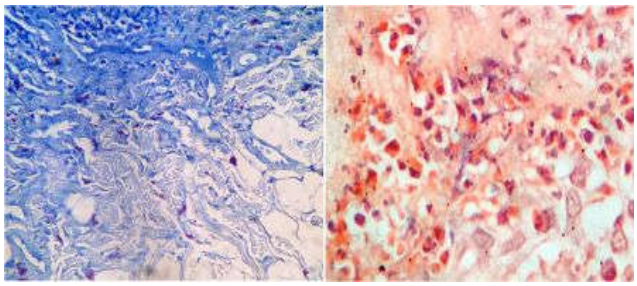

Figure 5 Histopathological picture showing mast cells and eosinophils interaction in dysplasia and stromal invasion during carcinoma process (40x).

In the present study, the eosınophıl counts showed an increasingly significant correlation in progression of dysplasia while mast cells did not show any correlation with such progression. While at the invasive fronts in oral carcinomas, mean mast cell counts showed a significant correlation while no such correlation is observed on observing eosinophils. Similar findings were reported by Tadbir AA *et al* 2009,[12] Joshi PS and Kaijkar MS 2013[13] also found no significant correlation between eosinophilic infiltration and histologial grades of SCC. Thus, we can suggest that eosinophils may play an important role in the progression of epithelial dysplasia into carcinoma but higher mast cells count in oral squamous cell carcinoma as compared to dysplasia suggested that they might have a role in stromal invasion.

CONCLUSION

In conclusion, eosinophils play a pivotal role in the histopathological progression of dysplasia. In addition, we observed higher mast cell counts in oral squamous cell carcinoma compared to dysplasia group justifying that they might have a role in stromal invasion. So, the present study recommends that quantitative assessment of eosinophils could become a part of the routine histopathological diagnosis for the oral premalignant disorders and mast cells helps in predicting the overall biological behaviour of oral squamous cell carcinoma.

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