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Original Research Article

Quantitative Estimation of Eosinopils in Dysplasia and Squamous Cell Carcinoma of Oral Cavity

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Abstract

Background: The tumor is not just malignant proliferating cancerous cells, but it has many other accompanying cells such as fibroblasts, macrophages, mast cells and NK cells that together form a complex tumor microenvironment (TME). These tumor cells along with TME form self-governing and self-sufficient biological structure.

Objective: to quantify the eosinophil infiltration in dysplastic and malignant lesions that is squamous cell carcinoma of oral cavity and its variation in various grades of dysplasia and squamous cell carcinoma of oral cavity.

Methods: This prospective cross-sectional study was done on tissue sections of clinically suspected and histologically proven cases of dysplastic and malignant lesions of oral cavity. The tissue studied were received in Histopathology section of the Department of Pathology, BLDE (Deemed to be university) Shri B M Patil Medical College Hospital & Research Centre, Vijayapura. Duration of study wasc 1st December, 2019 – 31st July, 2021.

Result: Mean age was 45.5 yrs. Male: female ratio of 1.96: 1 with 53 male cases and 27 female cases were noted in present study. A non-significant increase ($p>0.05$) was observed in the eosinophil count in squamous cell carcinoma when compared to epithelial dysplasia. Amongst the 32 cases of Epithelial dysplasia, increase in eosinophil count was observed in mild dysplasia with high standard deviation when compared to moderate and severe dysplasia. This increase eosinophil count was not statistically significant. ($p>0.05$). In 48 cases of squamous cell carcinomas studied, increased eosinophil count in well differentiated squamous cell carcinoma was found to be non- significant ($p>0.05$) when compared to moderately and poorly differentiated squamous cell carcinoma.

Conclusion: Eosinophil count was higher in SCC as compared to epithelial dysplasia. Thus multicentric, extensive studies with large sample size may help to consider role of eosinophils as a novel target for therapeutic agent.

Keywords: Estimation Of Eosinopils, Dysplasia, Squamous Cell Carcinoma, Oral Cavity.
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INTRODUCTION

The mechanism of recruitment and activation of eosinophils is brought mainly by inflammatory cytokines such as IL-4 and IL-13 that results in T helper cell mediated responses. Eosinophil activation involves chemical mediators like histamine, eotaxin and eosinophilic chemotactic factor A in mast cells, neutrophil peptides, C5a Complement etc. Even though eosinophils are commonly encountered in malignancies, their functional role remains indistinct[3]

The tumor is not just malignant proliferating cancerous cells, but it has many other accompanying cells such as fibroblasts, macrophages, mast cells and NK cells that together form a complex tumor microenvironment (TME). These tumor cells along with TME form self-governing and self sufficient biological structure. [1]

Tumor-associated tissue eosinophilia (TATE) was first described by Przewoski in cases of cervical carcinoma. TATE is defined as "eosinophilic stromal infiltration of a tumor not associated with tumor necrosis or ulceration."[2]

TATE has been studied in various cancers. Eosinophils estimation present in the surrounding inflammatory stroma of tumor tissue has been identified as a significant prognostic indicator in malignancies of breast, oesophagus, lung, intestine

and genitourinary tract. Its role may open new approaches toward the treatment.[3]

In various studies conducted on eosinophil count in oral lesions, it was found that the count is significantly high in oral malignancies as compared to dysplasia. Metastatic carcinomas had lower cell count that that of non-metastatic carcinomas. Based on this, esinophilia was concluded as a favourable histopathological prognostic factor in oral malignancies.[2,3,4]

A study done by Megha jain *et al*[2] showed significanty high mean eosinophil count in OSCC cases when compared with oral dysplasia cases, suggesting that they might have a role in stromal invasion. These authors also mentioned that their study findings are correlating with other studies in which it was mentioned that invasive squamous cell carcinoma has higher eosinophil count as compared to noninvasive tumors of head and neck region.

There is emerging evidence that show stromal components in tumor tissue play a significant role in the regulation of the Head and neck squamous cell carcinoma. The study of effect of tumor microenvironment and their role on cancer development and progression helps in defining possible targets for clinical intervention[1].

Hence this study will be undertaken to quantify the eosinophil infiltration in dysplastic and malignant lesions that is squamous cell carcinoma of oral cavity and its variation in various grades of dysplasia and squamous cell carcinoma of oral cavity.

Material and Methods

This prospective cross-sectional study was done on tissue sections of clinically suspected and histologically proven cases of dysplastic and malignant lesions of oral cavity. The tissue studied were received in Histopathology section of the Department of Pathology, BLDE (Deemed to be university) Shri B M Patil Medical College Hospital & Research Centre, Vijayapura. Duration of study wasc 1st December, 2019 – 31st July, 2021.

Sample Size Calculation: With 95% confidence level and margin of error of ±11%, a sample size of 80 subjects was taken to study the quantitative estimation of mast cells in oral epithelial dyslasia and oral squamous cell carcinoma. Total sample is 80 (32 cases of dysplasia and 48 cases of squamous cell carcinoma)

By using the formula:

$$n = \frac{z^2 p(1-p)}{d^2}$$

where
Z= z statistic at 5% level of significance.
d is margin of error.
p is anticipated prevalence rate of malignant samples.

Inclusion Criteria: Clinically suspected and histologically diagnosed cases of dysplasia and squamous cell carcinoma of oral cavity were included.

Exclusion Criteria: Cases of dysplasia and squamous cell carcinoma of oral cavity with other systemic diseases such as allergic conditions, parasitic infections and patients on corticosteroids were excluded.

Methods of Collection of Data

The study sample constituted of tissue sections of histopathologically diagnosed dysplastic and malignant lesions of oral cavity. The specimens received were fixed in formalin as per standard time required for fixation. The tissue sections were then processed in semiautomated tissue processor by routine procedure of tissue processing. Dehydration was done in graded alcohols (70%, followed by 95% and 100% solutions), followed by clearing in xylene. After processing, the tissue sections were impregnated with paraffin and blocks were prepared. Two serial sections each of 5µm thickness were prepared from these paraffin blocks using microtome and picked up on adhesive coated glass slides from water bath. One slide was stained with H&E and the other slide was stained with Toluidine blue stain.

Hematoxylin & Eosin (H&E) Staining: Staining was done as per standard protocol for quantitative evaluation of eosinophils.

Toluidine Blue Staining: Quantitative evaluation of mast cells was done on toluidine blue stained slide. Toluidine blue staining was done as per standard procedure.

Method of Counting of Eosinophils: Quantitative evaluation of eosinophils was done on H&E stained slide. Eosinophils were identified as bright red-to-orange granules containing cells that with basophilic-lobed nucleus. Eosinophil counts were done as per the study done by Jain *et al*[4]. Each tissue specimen slide was viewed in modified zig-zag pattern[4], without overlap under 10 consecutive high power that is 400X (under Labomed 500x microscope). The slides were examined by two observers independently to prevent interobserver bias. No exchange of information was done between both observers. Observations made by each observer regarding number of eosinophils under 400X magnification in 10 consecutive fields were recorded separately and average value is calculated for both observations. Eosinophils cell counts was done in cellular areas on tissue sections. The areas showing tissue necrosis, tissue folds and calcifications were not considered for counting.

Statistical Analysis: All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean±standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries and diagrammatic presentation. Chi-square ($\chi 2$) test was used for association between two categorical variables. The difference of the means of analysis variables between more than two independent groups was tested by ANOVA and F test of testing of equality of Variance.

Bivariate correlation analysis using Pearson's correlation coefficient (r) was used to test the strength and direction of relationships between the interval levels of variables. f the p-value was < 0.05, then the results were considered to be statistically significant otherwise it was considered as not statistically significant. Data were analyzed using SPSS software v.23(IBM Statistics, Chicago, USA) and Microsoft office 2007.

Results

The minimum age of the patient was 16 years and maximum were 75 years with the maximum number of cases in the range of 31-50 years amounting to 51.9%. Mean age was 45.5 yrs. The gender preponderance was found to be skewed towards male. Male: female ratio of 1.96: 1 with 53 male cases and 27 female cases were noted in present study.

Table 1: Distribution of Cases According to Site of Involvement

Site of Lesion	Number of Cases	Percentage of Cases
Tongue	37	46.3%
Lip	9	11.3%
GB sulcus	8	10%
Buccal mucosa	5	6.3%
Floor of mouth	3	3.8%
Hard palate	3	3.8%
Total	80	100%

In the present study, tongue was found to be most common site of involvement in dysplastic and malignant lesions of oral cavity with 37 cases amounting to 46.3%. This was followed by lip with 9 cases amounting to 11.3%.

The commonest clinical presentation in the present study was Proliferative growth with 29 cases amounting to 36.3%, followed by ulcerative lesion with 27 cases amounting to 33.8%.

Amongst 70 cases of lesions of oral cavity, 48 cases were histopathologically diagnosed as Squamous cell carcinoma (60%) and 32 cases were diagnosed as epithelial dysplasia (40%).

Table 2: Distribution of Cases According to Various Grades of Epithelial Dysplasia (N=32)

Dysplasia	Number	Percentage
Mild Dysplasia	19	59.4%
Moderate Dysplasia	10	31.3%
Severe Dysplasia	3	9.4%
Total	32	100.0%

Out of 32 cases of Epithelial dysplasia diagnosed on histopathology, maximum cases were of mild dysplasia amounting to 19 cases (59.4%). 10 cases were of moderate dysplasia amounting to 31.3% and 3 cases were of severe dysplasia amounting to 9.4%.

Table 3: Distribution of Cases According to Various Grades of Squamous Cell Carcinoma (N=48)

Squamous Cell Carcinoma	Number	Percentage
Well differentiated Squamous cell carcinoma	18	37.5%
Moderately differentiated Squamous cell carcinoma	27	56.3%
Poorly differentiated squamous cell carcinoma.	3	6.3%
Total	48	100.0%

Out of 48 cases of squamous cell carcinoma diagnosed on histopathology, maximum cases were of moderately differentiated Squamous cell carcinoma amounting to 27 cases (56.3%). 18 cases were of well differentiated Squamous cell carcinoma amounting to 37.5% and 3 cases were of severe dysplasia amounting to 6.3%.

Table 4: Quantitative Estimation of Average Eosinophil Count Between Epithelial Dysplasia and Squamous Cell Carcinoma. (N=80)

Average Eosinophils Per HPF	Dysplasia		SCC		p value
	Mean	SD	Mean	SD	
Observer 1	2.59	7.43	2.89	5.29	0.832
Observer 2	2.66	7.68	3.25	6.13	0.701
Average	2.62	7.55	3.07	5.70	0.762

In the present study, while evaluating the eosinophil count in the 80 cases of dysplastic and malignant lesions of oral cavity, a non-significant increase ($p>0.05$) was observed in the eosinophil count in squamous cell carcinoma when compared to epithelial dysplasia.

Table 5: Quantitative Estimation of Average Eosinophil Count in Various Grades of Oral Epithelial Dysplasia (N=32)

Average Eosinophils Per HPF	Dysplasia						p value
	Mild		Moderate		Severe		
	Mean	SD	Mean	SD	Mean	SD	
Observer 1	4.04	9.42	0.56	1.21	0.17	0.06	0.422
Observer 2	4.22	9.74	0.43	0.90	0.20	0.10	0.394
Average	4.13	9.58	0.50	1.06	0.18	0.08	0.407

Amongst the 32 cases of Epithelial dysplasia, increase in eosinophil count was observed in mild dysplasia with high standard deviation when compared to moderate and severe dysplasia. This increase eosinophil count was not statistically significant. ($p>0.05$)

Table 6: Quantitative Estimation of Average Eosinophil Count in Squamous Cell Carcinoma of Oral Cavity (N=48)

Average Eosinophil Count Per HPF	Squamous Cell Carcinoma						p value
	Well differentiated		Moderately differentiated		Poorly differentiated		
	Mean	SD	Mean	SD	Mean	SD	
Observer 1	5.19	7.75	1.66	2.30	0.27	0.31	0.057
Observer 2	5.98	9.14	1.75	2.19	0.40	0.52	0.050
Average	5.59	8.43	1.70	2.24	0.33	0.41	0.053

In 48 cases of squamous cell carcinomas studied, increased eosinophil count in well differentiated squamous cell carcinoma was found to be non-significant ($p>0.05$) when compared to moderately and poorly differentiated squamous cell carcinoma.

Table 7: Site Wise Quantification of Mean Eosinophil Count (N=80)

Site of Lesion	Observer	Dysplasia		SCC		p value
		Mean	SD	Mean	SD	
Buccal mucosa	Observer 1	2.14	5.60	1.31	2.26	0.716
	Observer 2	2.08	5.46	1.36	2.08	0.743
	Average	2.11	5.53	1.34	2.17	0.729
Floor of mouth	Observer 1	0.10	0.00	7.00	0.00	-
	Observer 2	0.10	0.00	8.00	0.00	-
	Average	0.10	0.00	7.50	0.00	-
GB sulcus	Observer 1	0.10	0.14	4.80	9.49	0.531
	Observer 2	0.10	0.14	5.30	10.25	0.521
	Average	0.10	0.14	5.05	9.87	0.526
Hard palate	Observer 1	0.10	0.00	4.35	5.02	0.615
	Observer 2	0.10	0.00	4.50	4.95	0.600
	Average	0.10	0.00	4.43	4.99	0.608
Lip	Observer 1	5.08	9.95	6.68	8.01	0.796
	Observer 2	5.53	10.98	7.64	10.03	0.772
	Average	5.30	10.47	7.16	9.00	0.782
Tongue	Observer 1	3.43	10.39	1.92	3.90	0.518
	Observer 2	3.54	10.70	2.21	4.75	0.601
	Average	3.49	10.55	2.06	4.31	0.560

Among the dysplastic lesion, lip showed highest levels of eosinophils 5.30 ±10.47 and among malignant lesion, squamous cell carcinoma from floor of mouth showed highest number of eosinophils 8.00 ±0 when compared to other locations within oral cavity. However, this increase was not statistically significant (p>0.05)

Discussion

The age group of the patients in our study ranged from 10-77 years. Maximum number of cases were in the range of 31-50 years amounting to 53.75%. In a study done by Kumar GK *et al.*[5] mean age of patients with dysplasia and squamous cell carcinoma was 46.87 years.

The gender preponderance was found to be skewed towards male, having male: female ratio of 1.96:1 with 53 male cases and 27 female cases. Our findings of male preponderance are corelating with studies done by Kumar GK *et al.*[5] & Ajay PR *et al.*[6] In their study male to female ratio was 2.28:1 and 2:1 respectively.

In our study, tongue was the commonest site of involvement in 46.3% of cases followed by buccal mucosa with 24.8% cases. Similar observation was found in a study done by Ahmad P *et al.*[7] wherein tongue was the most common tumor site for OSCC in 35.5% of the population.

Proliferative growth (seen in 36.3% cases) was the commonest clinical presentation in our study followed by ulcerative lesion (33.8% cases). Ulceroproliferative growth was seen in 27.5% cases and leukoplakia was observed in 2.5% cases. Misra *et al.*[8] found ulceration as the most common clinical presentation.

In the present study 32 cases (40%) cases were diagnosed as dysplasia. Incidence of mild, moderate and severe dysplasia was 59.4%, 31.3& and 9.4% respectively, which clearly suggests that majority of the patients were diagnosed to have mild dysplasia. This is in correlation with the study done on 630 cases of OED by Jaber MA. *et al.*[9]

On histopathology of 48 cases of squamous cell carcinoma, maximum cases were moderately differentiated squamous cell carcinoma amounting to 56.3%, followed by well differentiated squamous cell carcinoma and poorly differentiated squamous cell carcinoma amounting to 37.5% and 6.3% cases respectively. Similar results were seen in study conducted by Ayaz B *et al.*[10] wherein 43.28% cases were well differentiated, 47.76% cases were moderately differentiated, and 5.97% cases were poorly differentiated.

The average eosinophil count showed a non-significant increase in squamous cell carcinoma compared to epithelial dysplasia in our study. In contrast, Pereira *et al.*[11] found a significant increase in eosinophil count in OSCC (Mean± SD =17.84±5.4) as compared to premalignant disorders (Mean± SD = 6.4±1.5) (P= 0.0001). Similarly, studies done by Jain M *et al.*[2] and Deepthi G *et al.*[12] also found a significant increase in eosinophil count in OSCC as compared to dysplasia. However, in our study there was an increase in eosinophil count in OSCC as compared to dysplasia but it was non-significant. Higher eosinophil counts in OSCC compared to epithelial dysplasia may suggest that eosinophils might have a role in stromal invasion of tumor cells . Pereira *et al.*[11] suggested to use routinely assess tissue eosinophilia for the histopathological diagnosis for oral precancer and OSCC.[11]

In cases of epithelial dysplasia, increase in eosinophil count was observed in mild dysplasia (4.13 ± 9.58) when compared to moderate (0.50 ± 1.06) and severe dysplasia (0.18 ± 0.08). However, this increase eosinophil count was not statistically significant due to high standard deviation. Jain M *et al.*[2] also did not observe any statistical significance when eosinophil counts were compared among different grades of dysplasia. These results are in contrast to the findings of Madhura MG *et al.*[13] who observed the mean eosinophil count to be higher in severe dysplasia as compared among mild (1.05±1.146), moderate (2.71±1.254) and severe dysplasia (6.4±7.403). In addition, they also found that the eosinophil count was directly proportional to the degree of epithelial dysplasia. However, raised eosinophil count was not seen in all cases of severe dysplasia. The authors further mentioned that there are 60% chances to see higher eosinophil count with increase in severity of epithelial dysplasia.[13]

In squamous cell carcinomas cases, increased average eosinophil count was found in well differentiated squamous cell carcinoma (5.59 ± 8.43). However, this increase was non-significant (p>0.05) when compared to average eosinophil count in moderately differentiated (1.70 ± 2.24) and poorly differentiated (0.33 ± 0.41) squamous cell carcinoma. Our findings are in accordance with Shrestha *et al.*[14] where the eosinophil count decreased from well differentiated to poorly differentiated OSCC with no significant difference between eosinophils and different grades of OSCC. Jain M *et al.*[2] also found significantly higher eosinophilic counts in well differentiated SCC (5.9 ± 2.98) as compared to moderately differentiated SCC (3.7 ± 1.41) in metastatic cancer (p<0.05), whereas the difference was statistically non-significant in non-metastatic cancer group (well differentiated 8.2 ± 2.23 versus moderately differentiated 9.82 ± 3.4, p>0.05). In contrast to these studies, Deepthi G *et al.*[12] found a statistical significance in the eosinophil counts between well differentiated SCC (3.36 ± 1.4), moderately

differentiated SCC (4.43 ± 2.17) and poorly differentiated SCC (5.96 ± 2.19) with the number of eosinophils increasing from well to poorly differentiated squamous cell carcinoma. A study done by Kargahi N *et al.*[15] observed that the eosinophil count increases progressively from normal oral mucosa to OED and OSCC. They also found that eosinophil count increased from mild to severe dysplastic mucosa and from well differentiation to poor differentiation in SCC. The authors concluded that eosinophils can be regarded as an indicator of a developing malignancy along with other indicators, and they possibly can be used as a prognostic indicator for the disease. [15]

Tumor-associated tissue eosinophilia (TATE) is characterized by the presence of eosinophils as a component of peritumoural and intratumoural inflammatory infiltrate. The eosinophils are hypothesized to have direct tumouricidal activity with the release of cytotoxic proteins and they also act indirectly by enhancing the permeability into tumour cells facilitating penetration of tumour-killing cytokines.[16] Although presence of eosinophils has been demonstrated in oral cancer, their exact function or their prognostic value remains undefined.[17] It can be associated with poor prognosis or better prognosis or may even have no impact on the prognosis. It is postulated that eosinophils are like "Double edge sword in tumor battle field" that support cell mediated immunity in early stages but also inhibits cell mediated immunity in advanced stages via Indoleamine 2,3-Dioxygenase, which inhibit cytotoxic T cell responses.[16] In our study, eosinophil count decreased from well differentiated to poorly differentiated OSCC, which favours the protective role of eosinophils.

In our study 32 cases of Oral Epithelial dysplasia were studied for mast cell count between various grades of dysplasia. There was no significant relation ($p>0.05$) in average mast cell count per hpf by both observer 1 and observer 2 in various grades of dysplasia. Similar results were found in study done by Gomes AP *et al.* [18] where mean number of mast cells was not statistically different in mild dysplasia as compare to severe dysplasia. No significant difference in mast cell count was noted between normal mucosa, benign hyperkeratotic and dyskeratotic tissues by Jandinski *et al.*[19] . However, study done by Santana T *et al.*[20] showed that cases with a worse grade of epithelial dysplasia had a higher density of mast cells and this difference was statistically significant. We believe that this is due to the intrinsic characteristics of the samples and perhaps the intrinsic subjectivity to the grading systems of epithelial dysplasia.

Conclusion

There was a mild increase in eosinophil count in OSCC as compared to dysplasia, also there was a mild increase in eosinophil count in mild dysplasia as compared to moderate and severe dysplasia. Similarly increase in eosinophil count was seen in well-differentiated OSCC as compared to moderate and poorly differentiated OSCC. However, the difference was not statistically significant. Eosinophil count was high in well-differentiated OSCC as compared to poorly differentiated OSCC. Similar observations were noted in mild & severe dysplasia, which suggest that tissue eosinophilia might be a favourable histopathological prognostic factor in OSCC and dysplasia.

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