Research Article

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Tissue Eosinophilia as Histopathological Marker in Oral Potentially Malignant Disorders and Oral Squamous Cell Carcinoma

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ABSTRACT

Introduction: Oral potentially malignant disorders (OPMDs) and oral squamous cell carcinoma (OSCC) are common lesions. Correlation of tissue eosinophilia in OPMDs and OSCCs has shown varied results as a diagnostic and prognostic indicator.

Objective: To evaluate tissue eosinophilia in oral potentially malignant disorders and oral squamous cell carcinoma and its possibility as histopathological marker using Congo red stain.

Materials and Method: Analytical cross-sectional study in 100 histologically diagnosed cases of OPMDs, OSCCs and normal mucosa was done at Kantipur Dental College and Teaching hospital from Jan 2022 to May 2022. Convenience sampling technique was utilised. Cases and controls were stained with haematoxylin and eosin (H&E) and Congo red. Eosinophils were counted and recorded as eosinophils/10 hpf. Mean eosinophil count and mean difference between normal mucosa, OPMDs and OSCCs were compared and analysed using SPSS v.21.

Result: Mean difference in eosinophil count between different grades of dysplasia was statistically significant. There was statistically significant mean difference in eosinophil count between OSCC, dysplasia, OSMF, lichen planus, and normal mucosa. The mean eosinophil count was compared between H&E and Congo red stain in cases and control which showed significant difference.

Conclusion: Increased tissue eosinophilia from normal to OPMDs to OSCCs was found suggesting it might have role in stromal invasion.

Keywords: Eosinophils; oral potentially malignant disorders; oral squamous cell carcinoma.

INTRODUCTION

Eosinophils are immune cells having active role in diverse inflammatory responses including parasitic and helminthic infections, tissue injury, allergic diseases and tumour immunity.[1] Tumour associated tissue eosinophilia (TATE) has been observed in malignancies at different body sites including oral cavity.[2] Eosinophils may be tumouricidal associated with release of cytotoxic proteins. They may also promote tumour angiogenesis by the production of several angiogenic factors.[3,4]

Oral potentially malignant disorders (OPMDs) commonly comprises of oral leukoplakia, oral submucous fibrosis (OSMF), and oral lichen planus. Likewise, OSCC is the commonest malignant

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lesion. Correlation of tissue eosinophilia with the OPMDs and OSCCs has shown varied results as a diagnostic and prognostic indicator.[2,5]

Eosinophils, though can be identified under routine Haematoxylin and Eosin (H&E), sometimes may assume an uncommon morphology making them difficult to recognise. Hence, special stains like Sirius Red, Congo red, Luna, as well as modified H&E have been recommended for ease of ideintification.[5] Very few studies have been conducted to know the role of eosinophils in OPMDs. Hence, this study aims to elucidate the role of tissue eosinophilia in OPMDs and OSCCs and its possible use as a histopathological marker in these lesions using Congo red stain.

MATERIALS AND METHOD

An analytical cross-sectional study was carried out in the department of Oral and Maxillofacial Pathology, Kantipur Dental College and Teaching Hospital after ethical clearance. Histologically diagnosed cases of OPMDs and OSCC were taken from the archives of the Oral pathology from Jan 2022 to May 2022. Ethical approval was taken before conducting the study from Institutional Review Committee of Kantipur Dental College and Teaching Hospital (Ref. 33/021). In this study histologically diagnosed cases of Oral epithelial dysplasia, OSMF, Oral squamous cell carcinoma, Oral lichen planus, and healthy gingival mucosa from the extraction sites of impacted third molars which were free of inflammation were included. Normal gingival mucosa from the extraction sites of third molars were chosen because of ease of availability of the sample. Only nucleated cells with red cytoplasmic granules were accepted as eosinophils. Likewise red blood cells with superimposed mononuclear and polymorphonuclear inflammatory cells were excluded.

Convenience sampling method was used and the sample size was calculated using the formula: $n = Z^2 pq/e^2$; where, n = required sample size; Z = 1.96 at 95% confidence interval; p = 0.063 (prevalence of OSMF 6.3%);[6] q = 1-p; e = 0.05 (5% maximum permissible error). Hence, $n = 90.71 \approx 100$. Thus, the study consisted of five groups. The first group (Group I) comprising of 20 histologically diagnosed cases of Oral epithelial dysplasia, the second group (Group II) comprising of 20 histologically diagnosed cases of OSMF, the third group (Group III) comprising of 20 histologically diagnosed cases of OSCC, and the fourth group (IV) comprising of 20 histologically diagnosed cases of oral lichen planus. The fifth group (Group V) comprising of 20 gingival mucosa controls.

Eosinophils were counted in routine H&E as well as high pH Congo red stained slides. High pH Congo red stock solution was made using 0.3gm Congo red with 3 gm sodium chloride in 80% alcohol, working solution was made using 50 ml Congo red stock solution with 1% sodium hydroxide 0.5 ml. 3-4μm formalin fixed paraffin embedded tissue sections were obtained, deparaffinised, hydrated and placed in working Congo red solution for 10 minutes followed by washing in distilled water. Differentiation was done in 1% sodium hydroxide for five dips and counter stained with Harris haematoxylin for 30 seconds followed by water wash. Differentiation was done with ammonia water for 30 seconds, dehydrated in alcohol and cleared in xylene and mounted.[7]

Each specimen was viewed under high power (40X) microscopic field for counting eosinophils. Eosinophils were counted in 10 consecutive high-power field (hpf) by single observer and recorded as eosinophil/10 hpf.[1] For OSCC invasive front Figure 1 (a-h) whereas for OPMDs the connective tissue area was chosen for eosinophil count.

Data were transferred to Microsoft Excel Sheet and total eosinophil count between normal oral mucosa, OPMD, and OSCC were compared. Data was compared using independent t-test and correlation was done applying one-way Anova with Bonferroni correction using IBM SPSS Statistics for Windows, version 21 (IBM Corp., Armonk, N.Y., USA). P value of <0.05 with confidence level of 95% was set.

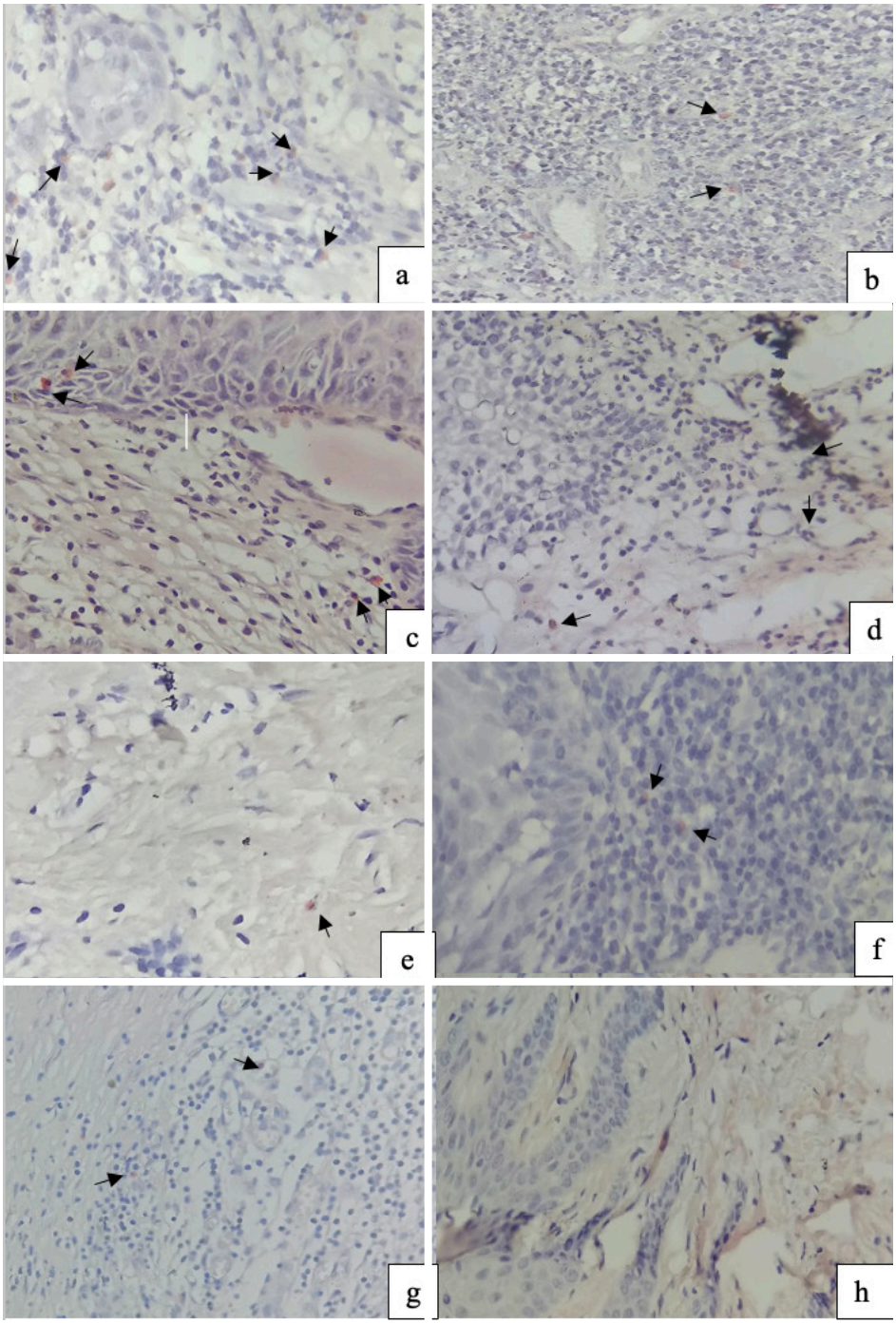

Figure 1: Eosinophils using Congo red stain in, a: Well differentiated OSCC; b: Moderately differentiated OSCC; c: Severe dysplasia; d: Moderate dysplasia; e: Mild dysplasia; f: Lichen planus; g: OSMF; and h: normal gingival mucosa.

RESULT

A total of 100 cases were evaluated for the study. The mean age of the study group was 45.39±14.61 years. Male were found to be more 63 (63%) compared to females 37 (37%). Buccal mucosa was the most common site 35 (35%) followed by gingiva 30 (30%).

The mean eosinophil count in dysplasia, OSMF, lichen planus, and normal gingiva is given in Table 1. Comparison of mean eosinophil count in well differentiated OSCC and moderately differentiated OSCC using independent t-tests, showed no statistically significant difference (P = 0.292, Table 2). Mean difference in eosinophil count using one-way Anova with Bonferroni correction between

Table 1: Total eosinophil count in oral potentially malignant disorders and normal control using congo red stain.

Group	N	Minimum	Maximum	Mean ± SD
Mild dysplasia	8	0	7	1.63 ±2.38
Moderate dysplasia	9	0	20	7.89±7.75
Severe dysplasia	3	10	27	19±8.54
OSMF	20	0	14	3.60±3.31
Lichen planus	20	0	3	0.90±1.11
Normal gingiva	20	0	2	0.80±0.76

Table 2: Comparison of eosinophil counts among different grades of oral squamous cell carcinoma using congo red and H&E stain.

Group	Eosinophil count	N	Grades	Mean ± SD	P value
OSCC	Using congo red	10	Well differentiated	12.80±8.854	0.29 (NS)
		10	Moderately differentiated	16.60±11.067	
	Using H&E	10	Well differentiated	4.80±4.492	0.35 (NS)
		10	Moderately differentiated	7.60±5.816	

Table 3: Comparison of eosinophil count among different grades of dysplasia using congo red stain.

Group	Eosinophil count	N	Grades		Mean difference	P value
Dysplasia	Using congo red	8	Mild	Moderate	-0.931	0.928 (NS)
				Severe	-5.042*	0.002
		9	Moderate dysplasia	Mild	0.931	0.928 (NS)
				Severe	-4.111*	0.011
		3	Severe dysplasia	Mild	5.042*	0.002
				Moderate	4.111*	0.011

* = The mean difference is significant at the 0.05

different grades of dysplasia showed a statistically significant difference of severe dysplasia with mild as well as moderate dysplasia (P <0.05), whereas there was no statistical significance difference between mild and moderate dysplasia (P = 0.928, Table 3).

The mean difference in eosinophil count using Congo red stain between dysplasia, OSMF, OSCC, lichen planus, and normal mucosa applying one-way Anova with Bonferroni correction showed significant difference between OSCC, OPMDs, and healthy controls (P <0.05, Table 4). When the mean eosinophil count was compared between Congo red and H&E in OPMDs, OSCC, and normal mucosa. A significant mean difference (P <0.05) was noted in all the cases as well as healthy controls except for lichen planus where in correlation and paired t-test cannot be computed since the standard error of the difference was 0 (Table 5).

Table 4: Comparison of total eosinophil count between epithelial dysplasia, OSMF, Lichen planus, OSCC and Normal mucosa using congo red stain.

Group		Mean difference	P value
Dysplasia	OSMF	3.450	0.738 (NS)
	OSCC	-7.650*	0.001
	Lichen planus	6.150*	0.017
	Normal mucosa	6.250*	0.015
OSMF	Dysplasia	-3.450	0.738 (NS)
	OSCC	-11.100*	<0.001
	Lichen planus	2.700	1.000 (NS)
	Normal mucosa	2.800	1.000 (NS)
OSCC	Dysplasia	7.650*	0.001
	OSMF	11.100*	<0.001
	Lichen planus	13.800*	<0.001
	Normal mucosa	13.900*	<0.001
Lichen planus	Dysplasia	-6.150*	0.017
	OSMF	-2.700	1.000 (NS)
	OSCC	-13.800*	<0.001
	Normal mucosa	.100	1.000 (NS)
Normal mucosa	Dysplasia	-6.250*	0.015
	OSMF	-2.800	1.000 (NS)
	OSCC	-13.900*	<0.001
	Lichen planus	-.100	1.000 (NS)

*. The mean difference is significant at the 0.05 level.

Table 5: Comparison of total eosinophil count between congo red and H&E stain in potentially malignant oral disorders and OSCC.

Group	N	Method	Mean±SD	t-value	P value
Dysplasia	20	Eosinophil count using congo red stain	-5.250±6.163	-3.809	0.001
	20	Eosinophil count using H&E stain			
OSMF	20	Eosinophil count using congo red stain	-1.850±2.681	-3.086	0.006
	20	Eosinophil count using H&E stain			
OSCC	20	Eosinophil count using congo red stain	-8.500±6.004	-6.331	<0.001
	20	Eosinophil count using H&E stain			
Normal mucosa	20	Eosinophil count using congo red stain	-.600±.503	-5.339	<0.001
	20	Eosinophil count using H&E stain			

DISCUSSION

Tumour stroma consists of various immune cells among which eosinophils are encountered many at times. The role of eosinophils in literature seems to be controversial as some consider it to be a favourable prognostic marker in OSCC, whereas some studies correlated it with poorer prognosis.[8,9] Hence, the present study was undertaken as an attempt to find out the role of tissue eosinophilia in OPMDs and OSCCs and whether it can be used as a histological marker in these lesions.

In the present study, mean age of study group was 45.39±14.61years, which further reinforces the concept that OPMDs and OSCC occurs in patients

older than 40 years.[10,11] In this study, similar to many other studies, there was a male preponderance (63, 63%) compared to females (37, 37%) and the most common site was buccal mucosa (35, 35%).[10,12]

In, the present study, eosinophil count between the severe, mild, and moderate dysplasia showed statistically significant (P <0.05) difference. Whereas there was no statistically significant difference in the eosinophil count between mild and moderate dysplasia (P = 0.928). This finding was similar to study by Madhura et al.[13] but was in contrast to study by Jain et al.[2] Madhura et al. in their study found that there were 60% chances to have higher eosinophil count with increase in severity of epithelial dysplasia. Hence, they have concluded that higher eosinophil count in dysplasia should be evaluated for invasiveness.[13]

In this study, invasive front of OSCC sample was chosen for eosinophils count. The peritumoural inflammatory infiltrates have been considered as the host's immune response. The initial activation and recruitment of eosinophils towards the tumour microenvironment is mediated by inflammatory cytokines and chemokines. This activation is considered principally related to Th2 response. IL-4 and IL-13 are potent chemokines that induces eotaxin hence, explaining the increase eosinophilia associated with Th2 response.[2] In the study, mean eosinophil count was more in moderately differentiated OSCC compared to well differentiated. Study by Siddiqui et al.[2] has shown increase in mean eosinophil count from well to moderately to poorly differentiated OSCC, implying that tissue eosinophilia was related to histological differentiation. In this study, no statistical difference in mean eosinophil count between the well differentiated and moderately differentiated grades of OSCC was noted (P = 0.292). This finding was similar to study by Tadbir et al.[14] and Jain et al.[2] Tadbir et al.[14] in their study reported no significant correlation between tissue eosinophilia and tumour differentiation in OSCC likewise study by Jain et al.[2] failed to show significant difference in mean eosinophilic count between well and moderately differentiated OSCC in non-metastatic cases.

In this study, comparison of the mean eosinophil count using special stain like Congo red with routine H&E showed statistically significant difference (P <0.05) in cases of dysplasia, OSMF, OSCC, and also normal gingiva mucosa control. Thus, use of special stains like Congo red can be of help for better analysis of eosinophils compared to routine hematoxylin and eosin stain.[15]

In this study, the mean eosinophilic difference between OSCC, dysplasia, OSMF, Lichen planus and normal mucosa was found to be highly significant (P <0.05) which was similar to study by Jain et al.[2] and Alrawi et al.[16] Jain et al. in their study have reported higher eosinophilic count in squamous cell carcinoma (SCC) than dysplasia, suggesting that it may have role in stromal invasion. Their study also concluded tissue eosinophilia as a favourable histopathological prognostic factor in OSCC.[2] Also, Alrawi et al.[16] reported increased eosinophil count in invasive SCC compared to noninvasive SCC concluding elevated eosinophilic count to be a histological marker associated with tumour invasion and also as a predictor for tumour aggressiveness.[16] Studies have suggested that increased eosinophilic infiltration may be induced by tumour-derived eosinophil chemotactic factor.[16,17]Also, a study has further indicated that stromal eosinophils in squamous cell carcinoma may play role in tumour invasion through activation of gelatinase. Eosinophil was found to express 92-kd gelatinase which may have role in tumour invasion by breaking down the basement membrane and extracellular matrix,[17] further advocating the role of eosinophil in invasion.

The results obtained from the present study showed that the number of eosinophils increased with progression of lesions from normal oral mucosa to oral potentially malignant disorders to OSCC. Hence increased tissue eosinophilia may act as a histological marker in OPMDs and OSCC. Since, this study was an initial attempt to assess tissue eosinophilia in dysplasia, OSMF, lichen planus and OSCC further studies with long term follow up is recommended to know the association of tissue eosinophila as prognostic indicator. Also, along with Congo red other special stains like carbol chromotrope may be used for eosinophil count.

CONCLUSION

In the present study, it was found that there was increase in tissue eosinophilia from normal to oral potentially malignant disorders to oral squamous cell carcinoma suggesting that it might have role in stromal invasion. Hence, higher eosinophilia may act as a histopathological marker in OPMDs and OSCC, also quantitative assessment of eosinophils ought to be part of histopathological diagnosis for these lesions.

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Conflict of interest: None.

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