Correlation of Mast cells and Microvessel densities in TNM staging, Grading and Nodal metastasis of Oral Squamous Cell Carcinoma (OSCC)

[1]Thirumalaraju Kalyani, [2]Dr. Yogesh Yadav, [3]Dr. Anju Lata Rai, [4]Dr. Devicharan Shetty
[1]PhD Scholar, Department of Anatomy, Santosh Medical College Deemed to be University, Ghaziabad, UP, India;
[2]HOD & Professor, Department of Anatomy, Noida International Institute of Medical Sciences, Greater Noida, UP, India;
[3]Professor, Department of Anatomy, ABVIMS, RML Hospital, New Delhi, India;
[4]Principal HOD & Professor, Department of Oral Pathology, ITS CDSR, Muradnagar, Delhi Meerut Road, Ghaziabad, UP, India

Corresponding author: Thirumalaraju Kalyani, PhD Scholar, Department of Anatomy, Santosh Medical College Deemed to be University, Ghaziabad, UP, India **Email:** tkalyani1412@gmail.com

Abstract

Background: Angiogenesis has been associated with tumor progression and metastasis of various malignancies including oral squamous cell carcinoma (OSCC). Among the host immune cells mast cellsare believed to secrete pro-angiogenic factors that may help in tumor metastasis and invasion. TNMstaging is important for treatment planning, estimating risk of recurrence, and assessment of overall survival of the patient. The aim of the present study was to examine the mast cell and microvessel withTNM staging and Lymph node metastasis in OSCC **Methods:** The study sample consisted of 220 positive OSCC sections were stained with routine haemotoxylin and eosin and included immunohistochemical staining anti-von Willibrand factor (vWF) for microvessels and toluidine blue for mast cells. **Results:** Our results showed a statistical significant correlation between MCD, MVD with grading (p<.0001) indicating significant increase from well to poorly differentiated squamouscell carcinoma. A significant positive correlation was seen between mast cell density (mm²) and microvessel density (mm²) in WDSCC (r=0.493, p value<.0001), MDSCC(r=0.442, p value=0.008), stage II(r=0.431, p value=0.0122), Stage IV (r=0.553, p value<.0001). In addition there was a statistically significant association of mean MVD in metastatic cases with clinical parameters (age, gender and siteof lesion) of OSCC. **Conclusion:** Further studies of large sample size of mast cell and microvessel with TNM staging, grading and nodal metastasis can be used as an additional parameter which would help in determining early therapeutic strategies like anti-angiogenesis therapy and survival rate in OSCC

Keywords: Tumor node metastasis, Micro vessel density, Mast cell density, Vascular endothelial growth factor.

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Introduction

Oral squamous cell carcinoma (OSCC) is an epithelial neoplasm with varying degrees of squamous differentiation that affect the tissues of the mouth. In 90% of oral cancers, squamous cell carcinomas are from mucous membranes of the mouth and oropharynx. India accounts high-risk for oral and oro-pharyngeal cancer due to a high prevalence of tobacco use, particularly chewing (in both sexes), smoking, and alcohol drinking in the male population[1].

There are many experimental and clinical data stating that angiogenesis aids in progression and metastasis in different malignant tumors including tumors of oral cavity.[2] Angiogenesis is stimulated by mast cells by release of angiogenic factors such as vascular

endothelial growth factor (VEGF), tumor necrosis factor (TNF), basic fibroblast growth factor (bFGF), histamine and heparin.[3]

Mast cells (MCs) are highly granulated cells present in the connective tissue and have a major role in type 1 hypersensitivity reaction[4,5].They are important in allergic reactions , inflammation,autoimmunity and t-cell mediated immune response and also involved in pain, tissue damage as well as repair[6]. Recent data suggests that the accumulation of mast cells around tumor margins and their release of pro-angiogenic factors may represent a tumor-host interaction which probably favors tumor progression[7-9] by promoting angiogenesis and degradation of extracellular matrix in some malignant tumors[10] . According to literature mast cell have been shown to increase the microvessel density (MVD) which in turn will play a role in producing lymph node metastasis[11].

With tumor progression the cancer cell penetrates through lymphatic endothelial cell barrier and finally drain into regional lymph nodes leading to lymphadenopathy. The occurrence of regional lymph node metastasis in OSCC is relatively high at 34%-50% [12]. There are many studies correlating mast cell and micro vessel density with different pathological grading but there is dearth of literature correlating mast cell density withangiogenesis in different stages of OSCC. Hence the aim of the present study was toevaluate mast cell and angiogenesis in OSCC by using toluidine blue to detect the mast cell density and vWF VIII marker to detect theMVD and to demonstrate its possible correlation with grading, staging and lymphnode metastasis.

Materials and Methods

The present retrospective study includes formalin-fixed paraffin embedded tissue blocks of 220 patients of histologically proven different grades of OSCC and associated lymph nodes (n=1310)

,10 normal oral mucosa (as controlled group) were archived from the department of oral and maxillofacial pathology during the period from 2019 to 2021. Relevant information like age,

gender, site of lesion, histopathological diagnosis were gathered from biopsy request form. The study was approved by the respective faculty of research and ethical committee of the institution. The paraffin blocks were sectioned on a rotatory semiautomatic soft tissue microtome into three sections 3µm each. Selected specimens were stained

1) Immunohistochemistry- A tissue section of 3 micron thickness were taken on poly-L-lysine coated glass slide for immunohistochemical staining with ready to use vWF VIII-Related antigen (Mouse monoclonal Antibody and poly-HRP (anti-mouse and anti-rabbit secondary antibody) (Biogenex Pvt.Ltd) for evaluation of MVD

2) 1% toluidine blue stain was performed for the evaluation of mast cell

3) 3µm thickness of representative tissue of lymph node block was obtained and stained with haematoxyline and eosine for the confirmation of nodal status.

Microvessel counting and morphometric assessement

The number of blood vessels were counted and assessed using Magnus pro image analysis software in four different high fields at x40. Microvessels were identified as dark brown vessels (Figure 1)and individual microvessels were marked along the outer border of endothelial cells of each blood vessel as “free hand area” and software generated results for each image were noted in millimeters.

Staining mast cells with toluidine blue- Tissue sections of 3µm were deparaffinized and hydrated to distilled water. Sections were stained in Toluidine blue working solution for 2-3 minutes. Washed in distilled water, 3

changes. Dehydrated quickly through 95% and 2 changes of 100% alcohol .Cleared in xylene and mounted with DPX.

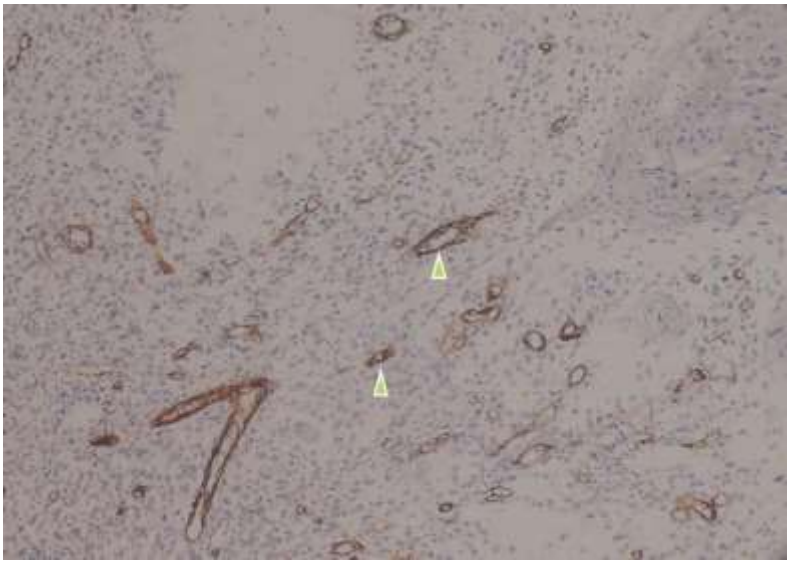
Mast cell counting- The stained slides were observed under x40 and mast cells were identified as magenta/purple colour granulocytes adjacent to tumor margins (Figure 2) and cells were counted in four different high fields.

Statistical analysis-

The data entry was done in the Microsoft EXCEL spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, ver 25.0 The presentation of the Categorical variables was done in the form of number and percentage (%). On the other hand, the quantitative data were presented as the means ± SD. The comparison of the variables which were quantitative in nature were analysed using Independent t test (for two groups) and ANOVA test (for more than two groups). Pearson correlation coefficient was used for correlation of Mast cell density(mm^2) and Micro vessel density (mm^2) and p value of less than 0.05 was considered statistically significant.

Results:

Figure 1: Light microscopic view with arrow mark showing number of vWF positive bloodvessels in moderately differentiated squamous cell carcinoma (10x)



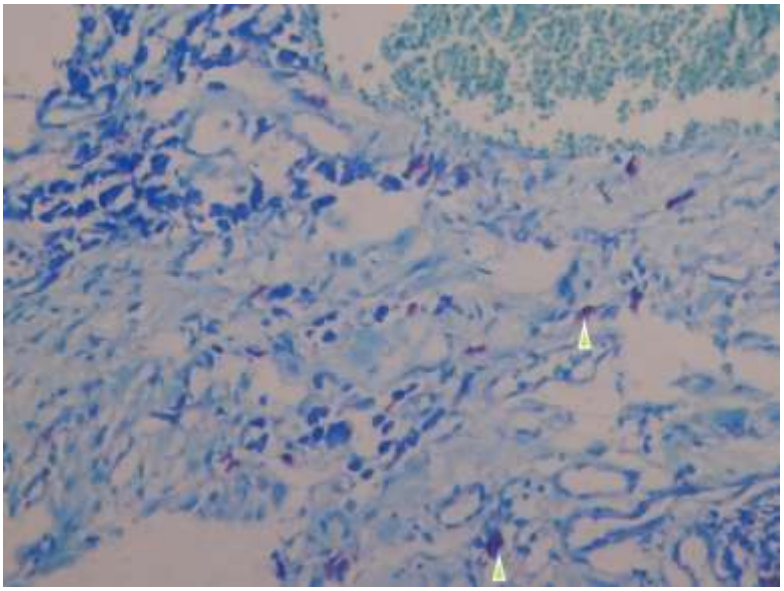


Figure 2: Light microscopic view with arrow mark showing mast cells stained with toluidine blue inmoderately differentiated squamous cell carcinoma (10x)

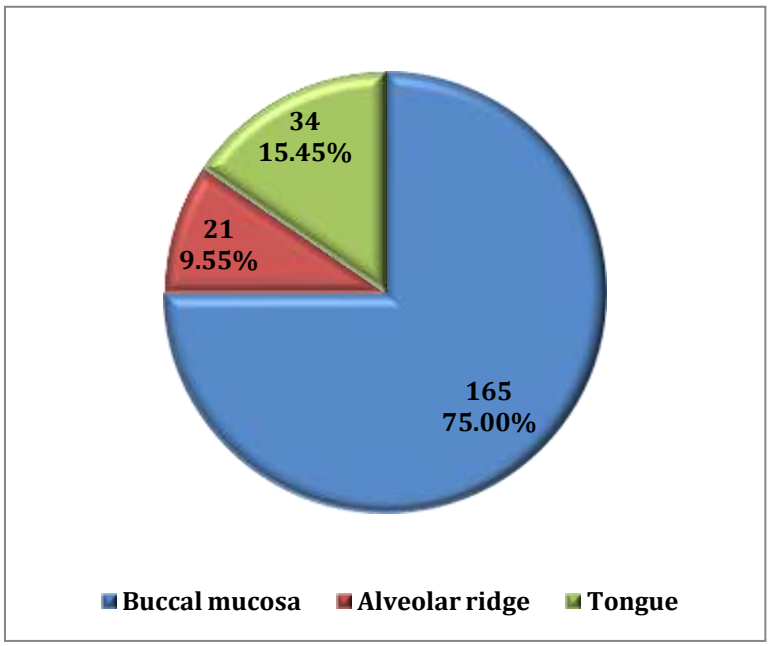


Figure 3:-Site wise distribution of oral squamous cell carcinoma

Buccal mucosa (75%) was the commonest site followed by tongue (15.45%) and alveolar ridge (9.55%)

Mean ± SD of mast cell density (mm²) and micro vessel density (mm^2) in PDSCC was significantly higher as compared to WDSCC and MDSCC p value<.0001 which was highly significant. (Table 1)

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Table 1:- Association of parameters with different grades of OSCC.

Parameters	WDSCC (n=145)	MDSCC (n=35)	PDSCC (n=40)	Total	P value
Mast cell density (mm^2)Mean ± SD	5.63 ± 2.73	7.54 ± 2.92	8.88 ± 3.46	6.52 ± 3.18	<.0001[§]
Micro vessel density (mm^2)Mean ± SD	15.16 ± 3.37	26.07 ± 7.22	44.51 ± 6.33	22.23 ± 12.2	<.0001[§]

[§] ANOVA, WDSCC- Well differentiated squamous cell carcinoma, MDSCC- Moderately differentiated squamous cell carcinoma and PDSCC- Poorly differentiated squamous cell carcinoma

Mean ± SD of mast cell density (mm^2) and micro vessel density in stage II was 6.78 ± 3.36 (p value=0.882) and 24.39 ± 12.75 (p value=0.176) as compared to stage III and stage IV with no significant association between them. (Table 2)

Table 2:- Association of mast cell density (mm^2) and Micro vessel density (mm^2) with TNM staging.

Parameters	stage II (n=33)	stage III (n=43)	stage IV (n=144)	Total	P value
Mast cell density (mm^2) Mean ± SD	6.78 ± 3.36	6.47 ± 3.58	6.48 ± 3.02	6.52 ± 3.18	0.882[§]
Micro vessel Density (mm^2) Mean ± SD	24.39 ± 12.75	19.4 ± 10.52	22.59 ± 12.46	22.23 ± 12.2	0.176[§]

[§] ANOVA

Significant positive correlation exist between mast cell density (mm^2) and micro vessel density (mm^2) in WDSCC(r=0.493, p value<.0001), MDSCC(r=0.442, p value=0.008), stage II (r=0.431, p value=0.0122), Stage IV (r=0.553, p value<.0001) which was highly significant. Table 3

Table 3:- Correlation of Mast cell density (mm^2) and Micro vessel density (mm^2).

Variables	Mast cell density (mm^2) and Micro vessel density(mm^2)	
	r	p value
Grading		
WDSCC	0.493	<0.0001
MDSCC	0.442	0.008
PDSCC	-0.106	0.517
Staging		
Stage II	0.431	0.0122

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Stage -III	0.281	0.0677
Stage-IV	0.553	<0.0001

WDSCC- Well differentiated squamous cell carcinoma, MDSCC- Moderately differentiated squamous cell carcinoma and PDSCC- Poorly differentiated squamous cell carcinoma

There was a statistical significant difference of MVD with and without metastasis when compared withage and gender. A higher statistically significant MVD in oscc of buccal mucosa was found when compared to oscc of alveolar ridge and tongue p<0.0003. (Table 4)

Table 4:- Association of micro vessel density (mm²) with various clinical parameters.

Variables	Metastasis	Non metastasis	Total	P value
Age				
Below 40 years	27.52 ± 14.48	17.08 ± 6.4	25.3 ± 13.82	0.0004‡
41 to 80 years	23.39 ± 12.49	18.24 ± 9.1	21.06 ± 11.34	0.003‡
Gender				
Male	22.8 ± 12.31	18.95 ± 9.38	21.5 ± 11.52	0.024‡
Female	36.06 ± 13.32	16.16 ± 6.87	24.86 ± 14.18	<.0001‡
Site				
Alveolar ridge	18.17 ± 14.32	16.08 ± 3.53	16.68 ± 7.81	0.739‡
Buccal mucosa	23.63 ± 12.29	17.83 ± 8.05	21.42 ± 11.2	0.0003‡
Tongue	30.98 ± 15.3	24.43 ± 17.54	29.63 ± 15.73	0.334‡

‡ Independent t test

Discussion:

Angiogenesis is considered to be a fundamental event in tumor progression and metastatisation and is regulated by numerous endogenous factors that stimulate or inhibit neovascularization. Among the host cells, which produce and release in a considerable quantity pro-angiogenic and angiogenic factors are mast cells .[13-15]

The present study was an attempt to correlate the mast cells, micro vessels with different histological grading, staging and nodal status.72.27% of the patients were in the age group of 41- 80 years indicating increase in the malignancy of older age group. Increase in mast cell density is associated with poor progression in different malignant tumors indicating their role in tumor progression.[16,17]In oral squamous cell carcinoma mast cells have been identified through several histochemical stains like toluidine blue, alcian blue and immunohistochemically by mast cell tryptase, heparine and chymase[18]. In the present study MC count was evaluated by 1% toluidine blue (Figure 2) and mast cell density was found to be significantly increased in different grades of OSCC (p<.0001). Micro vessel density was evaluated by using anti factor VIII related antigen (vWF) (Figure 1). MCD and MVD were found to be increased in different grades of OSCC (p<.0001) (Table 1)

There was no significant correlation between MCD (p<0.882), MVD (p<0.176) with TNM staging.(Table2). MCD and MVD were

correlated in different grades of OSCC and TNM staging and found a significant correlation in WDSCC (r= 0.493, p<0.05) and MDSCC (r=0.442, p<0.008) which indicates that mast cells are one of the important angiogenic factors promoting tumor angiogenesis[4,19]. There was a significant positive correlation between MCD and MVD in stage II (r=0.431, p<0.012) and stage IV (r=0.553,p<0.05) , but not in stage III (Table 3), suggesting degranulation of mast cells in stage III samples making it difficult to count mast cells.[20-22]

Similar to our study Pargat singh T et al[23] found a significant positive correlation between MCD and MVD suggesting that mast cells aid in the upregulation of tumor angiogenesis as they help in secretion of angiogenic factors like histamine, heparin, tryptase and VEGF. In contrast to our study Kalra et al,[24] on mast cell and blood vessel density in different grades of OSCC found significant increase in blood vessel density whereas mast cell density decreased from WDSCC to PDSCC. A significant but inverse correlation was seen in their study attributing to a migration failure of mast cells during tumor initiation and progression.[9]

Nodal metastasis is very important contributing factor of prognosis and is more frequently encountered than distant metastasis.[25-27] Much in advance to the invasion of LN by the tumor, the LN reacts to numerous molecules produced to the response of the tumor. Regional lymph nodes in OSCC are considered to be the primary immunological defense mechanism against tumors.[28] Invasion and metastasis are the most insidious and life threatening aspects of cancer. In the present study higher mean mast cell and microvessel density were found in OSCC patients with nodal metastasis and MVD was found to have a statistical correlation with age, gender and site of lesion (buccal mucosa) (Table 4).

Conclusion:

On the basis of our study, we can thus conclude that a significant correlation exist between MCD and MVD with progression of OSCC. From the prognostic point of view, designing studies to determine the relationship between MCD, MVD and survival rate in oral cancers is to be encouraged in larger samples, along with planning and developing of various adjuvants therapeutic strategies like anti-angiogenesis therapy and vascular targeting the anticancer therapy.

References:

1. Tandon P, Dadhich A, Saluja H, Bawane S, Sachdeva S. The Prevalence of squamous cell carcinoma in different sites of oral cavity at our rural health care center in loni, Maharastra- a retrospective 10-year study. Contemporary Oncology 2017; 21(2):178-183
2. Gaje PN, Amalia Ceausu R, Jitariu A, Stratul SI, Rusu LC, Popovici RA. et al. Mast Cells: Key Players in the Shadow in Oral Inflammation and in Squamous Cell Carcinoma of the Oral Cavity. Biomed Res Int. 2016; 2016:9235080
3. Kheur S., Neeta D. P., Kulkarni B. M., Routray S., Dhas V. Role of ast cell in oral pathology. *Oral & Maxillofacial Pathology Journal.* 2013;4(2):408–412.
4. Michailidou EZ, Markopoulos AK, Antoniades DZ. Mast cells and angiogenesis in oral malignant and premalignant lesions. Open Dent J 2008;2:126-32.
5. Conti P, Castellani ML, Kempuraj D, Salini V, Vecchiet J, Tete S, et al. Role of mast cells in tumor growth. Ann Clin Lab Sci 2007;315-22
6. Tahir A, Nagi AH, Ullah E, Janjua OS. The role of mast cells and angiogenesis in well-differentiated oral squamous cell carcinoma. J Can Res Ther 2013;9:387-91.
7. Gaje P, Bocon V, Cimpean AM, Izvernariu DA, Streian F, Raica M. Simultaneous demonstration of mast cells and blood vessels

by the combined method CD 34-alcian blue-safranin in lip tumors. Rom J Morphol Embryol 2007;48:237-41.

8. Ascani G, Balercia P, Messi M, Lupi L, Goteri G, Filosa A, Stramazzotti D, Pieramici T, Rubini C. Angiogenesis in oral squamous cell carcinoma. Acta Otorhinolaryngol Ital 2005;25:13-7.
9. Oliveira-Neto HH, Gleber-Netto FO, de Sousa SF, França CM, Aguiar MC, Silva TA, Batista AC. A comparative study of microvessel density in squamous cell carcinoma ofthe oral cavity and lip. Oral Surg Oral Med Oral Pathol Oral Radiol 2012;113:391-8.
10. Ch'ng S, Sullivan M, Yuan L, Davis P, Tan ST. Mast cells dysregulate apoptotic and cellcycle genes in mucosal squamous cell carcinoma. Cancer Cell Int 2006; 6:28.
11. Nakandala K, Suraweera A, Jayasooriya P. Correlation of elevated mast cell and micro-vessel densities with lymph node metastasis in oral squamous cell carcinoma. Stomatological Dis Sci 2018;2:4.
12. Noguti J, De Moura CF, De Jesus GP, Da Silva VH, Hossaka TA, Oshima CT, Ribeiro DA. Metastasis from oral cancer: an overview. Cancer Genomics Proteomics 2012;9:329-35
13. Jahanshahi G, Sabaghian M. Comparative immunohistochemical analysis of angiogenesis and mast cell density in oral normal mucosa and squamous cell carcinoma. Dental Research Journal. 2012;9(1):8-12.
14. Sharma B, Sriram G, Saraswathi TR, Sivapathasundharam B. Immunohistochemical evaluation of mast cells and angiogenesis in oral squamous cell carcinoma. Indian J Dent Res 2010;21:260 5.
15. Iamaroon A, Pongsiriwet S, Jittidecharaks S, Pattanaporn K, Prapayasatok S, Wanachantararak S. Increase of mast cells and tumor angiogenesis in oral squamous cell carcinoma. J Oral Pathol Med 2003; 32:195-9
16. Yodavudh S, Tangjitgamol S, and Puangsa-art S. "Prognostic significance of microvessel density and mast cell density for the survival of Thai patients with primary colorectal cancer" Journal of the Medical Association of Thailand, 2008; vol. 91(5):723–732,
17. Weller C.L, Collington S.J, Williams T, and Lamb J.R. "Mast cells in health and disease," Clinical Science. 2011; vol. 120, no. 11, pp.473–484.
18. Kabiraj A, Jaiswal R, Singh A, Gupta J, Singh A, Samadi FM. Immunohistochemical evaluation of tumor angiogenesis and the role of mast cells in oral squamous cell carcinoma. J Can Res Ther 2018;14:495-502.
19. Starke RD, Ferraro F, Paschalaki KE, Dryden NH, McKinnon TA, Sutton RE, et al.Endothelial von Willebrand factor regulates angiogenesis. Blood 2011;117:1071‑80.
20. Nakandala K, Suraweera A, Jayasooriya P. Correlation of elevated mast cell and micro-vessel densities with lymph node metastasis in oral squamous cell carcinoma. Stomatological Dis Sci 2018;2:4.
21. Mohseni MG, Mohammadi A, Heshmat AS, Kosari F, Meysamie AP. The lack of correlation between mast cells and microvessel density with pathologic feature of renal cellcarcinoma. Int Urol Nephrol 2010; 42: 109–112.
22. Aroni K, Tsagroni E, Kavantzas N, Patsouris E, Ioannidis E. A study of the pathogenesis of rosacea: How angiogenesis and mast cells may participate in a complex multifactorial process. Arch Dermatol Res 2008;300:125-31.
23. Kathuriya PT, Bartake AR, Palaskar SJ, Narang BR, Patil SS, Pawar RB. Cd34 and Mast Cell Analysis in Normal Oral Mucosa and Different Grades of Oral Squamous Cell Carcinoma: A Comparative Study. J Clin Diagn Res. 2015 Jul;9(7):ZC61-4.
24. Kalra M, Rao N, Nanda K, Rehman F, Girish KL, Tippu S, et al. The role of mast cells on angiogenesis in oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal 2012;17:e190-6
25. Gale N, Pilch BZ, Sidransky D, Westra WH, Califano J. Epithelial precursor lesions. In: Barnes L, Eveson JW, Reichart P, Sidransky D,

editors. World Health Organization Classification of Tumours. Pathology and Genetics of Head and Neck Tumours. Lyon: IARC Press; 2005. p. 177-80.

26. Jayasooriya PR, Pitakotuwage TN, Mendis BR, Lombardi T. Descriptive study of 896 oral squamous cell carcinomas from the only University based Oral Pathology Diagnostic Service in Sri Lanka. BMC Oral Health 2016;16:1.
27. Anuradha A, Kiran Kumar Naik B, Vijay Srinivas G, Devi RS, Puneet HK. Incidence of mast cells in oral squamous cell carcinoma: a short study. J Oncol 2014;2014: 614291.
28. Balla H, Uppala D, Majumdar S, Kotina S, Kodati S and Namana M. Evaluation of immunomorphological patterns of cervical lymph nodes in oral squamous cell carcinoma. J Oral Maxillofac Pathol. 2020;24(2):285-292

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