Original Article

Extracellular matrix metalloproteinase inducer expression in histological grades of oral squamous cell carcinoma – An immunohistochemical study

Sabiha Mohiddin Khan[1], Nilima Prakash[2], G L Pradeep[2], Aarti Mahajan[2], Rizwan Raiskhan Mokashi[3], Rekha Patil[2]

[1]Indian Cancer Society, Mumbai, [2]Department of Oral and Maxillofacial Pathology, MGV'S KBH Dental College and Hospital, [3]Department of Conservative Dentistry and Endodontics, SMBT Institute of Dental Sciences, Nashik, Maharashtra, India

Abstract

Background: Oral squamous cell carcinoma (OSCC) is the most common type of head-and-neck cancer. It is a complex and relentless malignancy prone to local invasion and dissemination. An insight into the molecular alterations associated with metastasis will provide critical insights into the fundamental mechanisms underlying its progression and further contribute to improvements in the clinical management of H and N cancer patients. Hence, identifying specific biomarkers would pave the way for early detection and prognosis of OSCC. Extracellular matrix metalloproteinase inducer (EMMPRIN) is a membrane-bound glycoprotein found on the surface of tumor cells. It plays a central role in the promotion of tumor invasion, progression and metastasis as it upregulates matrix metalloproteinases secreted from adjacent fibroblasts. There is a paucity of studies on the expression of EMMPRIN in OSCC.

Objectives: The aim is to assess the immunohistochemical expression of EMMPRIN in OSCC and to compare it with the clinicopathological parameters and histological grades of OSCC.

Materials and Methods: Thirty histopathologically diagnosed cases of OSCC were included in the study. The slides were immunohistochemically analyzed for EMMPRIN expression and correlated with the clinicopathological parameters and histological grades of OSCC.

Results: EMMPRIN expression was noted in all 30 cases of OSCC. Strong EMMPRIN expression was noted in the advanced clinical stages of OSCC. Higher histological grades of OSCC exhibited strong EMMPRIN expression.

Conclusion: EMMPRIN overexpression indicates that this protein could be used as an important biological prognostic marker to identify high-risk OSCC patients.

Keywords: Extracellular matrix metalloproteinase inducer, oral squamous cell carcinoma, prognosis

Address for correspondence: Dr. Nilima Prakash, Department of Oral and Maxillofacial Pathology, MGV'S KBH Dental College and Hospital, Nashik, Maharashtra, India.
E-mail: drnilimaprakash@gmail.com

Submitted: 23-Jan-2020, **Revised:** 29-Oct-2020, **Accepted:** 03-Nov-2020, **Published:** 09-Jan-2021

INTRODUCTION

Oral squamous cell carcinoma (OSCC) represents >45% of all malignancies reported in India and accounts for up to 30% of all new cases of cancer.[1] Approximately 90% of deaths due to oral cancer are caused by metastasis.[2]

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.JOMFP_26_20

How to cite this article: Khan SM, Prakash N, Pradeep GL, Mahajan A, Mokashi RR, Patil R. Extracellular matrix metalloproteinase inducer expression in histological grades of oral squamous cell carcinoma – An immunohistochemical study. J Oral Maxillofac Pathol 2020;24:530-5.

Late detection, low therapeutic response and metastasis are the foremost confounders accountable for the poor 5-year survival rate of OSCC.[3] New insights into cancer diagnosis and therapy have not changed the survival rate for oral cancer (around 50%) during the last decades.[4] Increased mortality rate could be attributed to the lack of specific biomarkers to predict tumor progression and prognosis of the patients.[5,6] Hence, identifying specific biomarkers would pave the way for early detection and better prognosis of OSCC.

Extracellular matrix metalloproteinase inducer (EMMPRIN), also known as CD147, is a membrane-bound glycoprotein found on the surface of tumor cells. Implicated in a variety of physiological and pathological activities, it is best known for its ability to function as inducer of matrix metalloproteinases (MMPs). It is a transmembrane protein that regulates the turnover, remodeling of the extracellular matrix and is an important mediator of cell and stromal interactions.[7]

Dysregulation of EMMPRIN has been linked to almost every type of cancer. There is evidence that EMMPRIN is central in the promotion of tumor invasion, growth/progression and metastasis as it upregulates MMPs secreted from adjacent fibroblasts. In addition, EMMPRIN influences the production of several proinflammatory cytokines that have been directly linked to cancer.[7] EMMPRIN also promotes neovascularization through the expression of vascular endothelial growth factor.[8]

Elevated EMMPRIN expression has been shown to correlate with lymphatic metastasis and tumor progression in most cancers. EMMPRIN is overexpressed to varying degrees in most tumor types, with head and neck squamous cell carcinomas having some of the highest levels of EMMPRIN expression.[9]

However, there is a paucity of studies on the immunoexpression of EMMPRIN in OSCC in the Indian subcontinent. Thus, the purpose of the present study was to assess the immunoexpression of EMMPRIN in OSCC and to correlate it with the clinicopathological parameters and histological grades of OSCC.

MATERIALS AND METHODS

The present study was a comparative study, and sampling was done by simple random sampling method. Ethical clearance was obtained from the institutional ethical committee and informed consent was obtained from patients for the present study.

The study group comprised 30 histopathologically diagnosed cases of OSCC. For control, normal oral mucosa samples were obtained during crown lengthening and disimpaction procedures.

Relevant information (viz. age, sex, site of the lesion, habit history, duration and frequency of habit) was recorded on the case history pro forma. Staging of OSCC was done according to the staging system suggested by the American Joint Committee for Cancer Staging and End Results Reporting known as the TNM system.[10] Two sections of 4-μm thickness each was obtained from the formalin-fixed, paraffin-embedded tissues. One section was stained with hematoxylin and eosin stain. Brynes' histological grading criteria were used to grade the cases of OSCC. Another section was placed on aminopropyltriethoxysilanecoated slide for immunohistochemical staining with EMMPRIN antibody (Monoclonal Purified Anti-Human CD-147, 1:200, Clone HIM6, Isotype-Mouse IgG1,κ, Biolegend Lab, San Diego, California) using NovolinkTM Polymer Detection System.

Evaluation of immunoexpression of extracellular matrix metalloproteinase inducer

For each case, nonoverlapping fields at ×400 were observed and 1000 epithelial cells at the invasive tumor front were counted. Cytoplasmic and membranous staining for EMMPRIN was accepted as positive. Each slide was evaluated according to the staining extent and intensity. Immunopositive cells were counted in the same fields. The assessment was made by semi-quantitative scoring method.[11]

Staining extent and intensity scores were added to obtain combined scores that were then allocated to 4 groups - 0–1 = Negative staining, 2–3 = Weak staining, 4–5 = Moderate staining and 6–7 = Strong staining.

Statistical analysis

The nonparametric Mann–Whitney U-test and Kruskal–Wallis ANOVA test were applied for the evaluation of significant differences among the mean values in different groups. Mann–Whitney U-test was applied to compare EMMPRIN scores with clinicopathological parameters and histological grades. Correlation between EMMPRIN expression and clinicopathological parameters were obtained by Pearson correlation test. Results with "$P < 0.05$" were considered to be statistically significant at a 95% confidence interval.

RESULTS AND OBSERVATIONS

Demographic details pertaining to age and gender were computed, majority of the patients with OSCC 20 (66.66%)

were in the age group <60 years with a mean age of 54.17 years. A definite male predominance was noted, accounting for 25 (83.33%) cases. On studying the site distribution of the lesion, buccal mucosa 20 (66.67%) was the predominant site [Table 1].

On TNM staging, majority of the patients belonged to Stage II, accounting for 10 cases (33.33%) followed by 9 (30.00%) patients in Stage I, 8 (26.67%) in Stage III and 3 (10.00%) in Stage IV [Table 2].

According to the invasion front grading system by Bryne's grading system, 13 (43.33%) cases of OSSC were categorized as Grade I (Well differentiated), 12 (40.00%) cases fell under the category of Grade II (Moderately differentiated) and 5 (16.67%) cases were Grade III (poorly differentiated) [Table 3].

Immunoexpression of extracellular matrix metalloproteinase inducer

Comparison of EMMPRIN expression with demographic data (age, sex and site) was done. The results were not statistically significant.

EMMPRIN expression in the clinical stages of OSCC was assessed. The advanced stages exhibited strong EMMPRIN expression. Stage IV showed strong EMMPRIN expression in all cases (100.00%). Strong expression was noted in Stage III (87.5%), Stage II (70%) and Stage I (44.44%). However, EMMPRIN overexpression and clinical stage of OSCC did not show significant association ($P = 0.2609$) [Table 2 and Graph 1].

On comparison of EMMPRIN expression in histological grades of OSCC, higher grades (MDSCC and PDSCC) showed strong expression of EMMPRIN in 12 (100.00%) and 5 (100.00%) cases, respectively. Only 4 cases (30.77%) of WDSCC cases showed strong expression. The EMMPRIN expression in different histological grades of OSCC was found to be statistically significant ($P = 0.0021$) [Table 3 and Graph 2].

Normal oral mucosa showed weak EMMPRIN expression localized to the basal layer of epithelium. This is consistent with the study done by Arora M and Mane DR.[12]

DISCUSSION

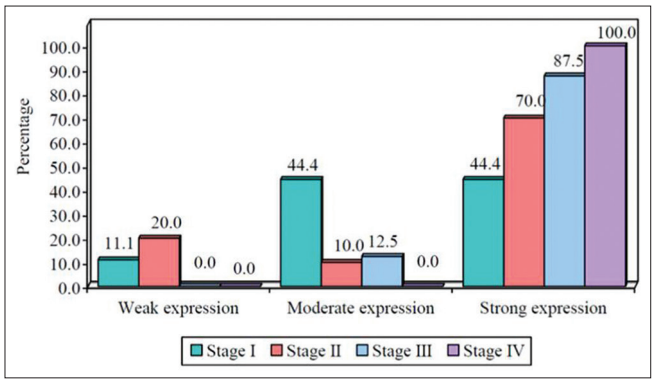
The oral cavity is one of the predominant sites of

Table 1: Immunoexpression of EMMPRIN according to age sex and site of OSCC cases

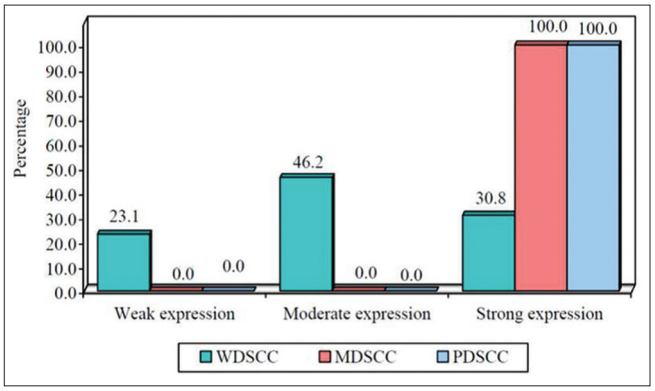
Parameter	EMMPRIN expression			*P*
	Weak	Moderate	Strong	
Sex				
Male	2 (8%)	5 (20%)	18 (72%)	0.7097
Female	1 (20%)	1 (20%)	3 (60%)	
Age				
<60yrs	3 (15%)	5 (25%)	12 (60%)	0.2115
≥61yrs	0 (0%)	1 (10%)	9 (90%)	
Site				
Buccal mucosa	3 (15%)	5 (25%)	12 (60%)	0.7104
Floor of Mouth	0 (0%)	0 (0%)	3 (100%)	
Tongue	0 (0%)	1 (20%)	4 (80%)	
Alveolus	0 (0%)	0 (0%)	2 (100%)	

Table 2: Immunoexpression of EMMPRIN according to Clinical Stages of OSCC cases

Parameter	EMMPRIN expression			*P*
	Weak	Moderate	Strong	
Tumor size				
T1	1 (9.09%)	5 (45.45%)	5 (45.45%)	0.2222
T2	2 (14.29%)	1 (7.14%)	11 (78.57%)	
T3	0 (0%)	0 (0%)	2 (100%)	
T4	0 (0%)	0 (0%)	3 (100%)	
Nodal involvement				
N0	3 (15.70%)	5 (26.32%)	11 (57.89%)	
N1+N3	0 (0%)	1 (9.01%)	10 (90.91%)	0.1463
TNM Stage				
Stage I	1 (11.1%)	4 (44.4%)	4 (44.4%)	
Stage II	2 (20%)	1 (10%)	7 (20%)	0.2609
Stage III	0 (0%)	1 (12.5%)	7 (20%)	
Stage IV	0 (0%)	0 (0%)	3 (70%)	



Graph 1: Immunoexpression of EMMPRIN in Clinical Stages of OSCC



Graph 2: Immunoexpression of EMMPRIN in histological stages of OSCC

Table 3: Immunoexpression of EMMPRIN according to Histological grades of OSCC cases

Parameter	EMMPRIN expression			*P*
	Weak	Moderate	Strong	
Histologic Grades				
WDSCC	3 (23.08%)	6 (46.15%)	4 (30.77%)	
MDSCC	0 (0%)	0 (0%)	12 (100%)	0.0021*
PDSCC	0 (0%)	0 (0%)	5 (100%)	

development of malignancies since it comes into direct contact with many carcinogens. Early detection of oral cancer is critical as it decreases the mortality and morbidity rate. At present, therapeutic decisions are based on clinicopathological parameters, including age, clinical stage and histological grades. Although useful, these factors often fail to provide accurate information regarding the biological behavior of the tumor. Therefore, an insight into the molecular alterations associated with OSCC will provide a better understanding of the fundamental mechanisms underlying its progression and further contribute to improvements in its clinical management.[13]

EMMPRIN or CD147 is a transmembrane glycoprotein[14] implicated in tumorigenesis, it is known to regulate cell proliferation, apoptosis, tumor cell migration, angiogenesis and metastasis, especially under hypoxic conditions.[7]

In this study, membranous and cytoplasmic staining for EMMPRIN was accepted as positive. The assessment was done by the semi-quantitative scoring method described by Kefeli *et al*.[11] Our study showed that EMMPRIN expression was present in all OSCC cases ranging from weak to strong expression. It was overexpressed in majority of the cases.

On the assessment of EMMPRIN expression in different age groups of OSCC, majority of patients, that is, 12 patients (60%) <60 years, showed strong expression and only 9 (90%) patients ≥60 years showed strong expression [Table 1]. However, there was no statistically significant difference between EMMPRIN expression and age groups.

Similarly, studies by Monteiro *et al*.[4] did not exhibit a statistically significant association of EMMPRIN expression with age.

Patient's age is a commonly considered co-variable and is known to influence the outcome of the treatment. Genetic susceptibility to environmental carcinogens may influence the risk of OSCC in young adults. While the correlation of age with prognosis seemed controversial, it is generally considered that OSCC in young people are less aggressive and have a good prognosis.[15]

EMMPRIN expression was compared with the gender of OSCC patients. We found that strong EMMPRIN expression was more common in male patients accounting for 18 cases (72.00%) compared to 3 cases (60%) in female patients. However, there was no statistically significant difference between EMMPRIN expression and gender ($P = 0.7097$) [Table 1].

This was consistent with the results of Monteiro *et al*.[4] who found higher EMMPRIN expression in male patients, which was not statistically significant.

There was an overall male predominance in OSCC of all intraoral sites in this study, most of which were advanced. Higher EMMPRIN expression may signal a more aggressive course.

On the assessment of EMMPRIN expression in OSCC at different sites, floor of the mouth and alveolus showed strong expression in 3 and 2 (100%) of cases, respectively. Tongue lesions showed strong expression in 4 (80%) OSCC patients and buccal mucosa lesions showed strong expression in 12 (60%) cases. However, there was no statistically significant difference between EMMPRIN expression and site of OSCC [Table 1].

In the study by Monteiro *et al*.,[4] highest EMMPRIN expression was seen in buccal mucosa, i.e., 100% followed by labial mucosa (85.7%) and tongue (62.5%). However, their findings were not statistically significant.

There is a strong association between the site of cancer and the site where the quid is placed regularly. Pooling of carcinogens in saliva gives rise to cancers in the floor of the mouth and ventral and lateral tongue. OSCC of the tongue and floor of the mouth is associated with poor prognosis. Higher EMMPRIN expression in OSCC of the floor of mouth and tongue may indicate an aggressive course.[16]

On the assessment of EMMPRIN expression in different clinical stages of OSCC, strong EMMPRIN expression was noted in advanced stages of OSCC (Stage II and III). However, EMMPRIN overexpression and clinical stage of OSCC did not show significant association ($P = 0.2609$) [Table 2 and Graph 1].

Similarly, Monteiro *et al*.[4] in their study found EMMPRIN overexpression in patients with advanced clinical Stages (III/IV). Their results were not statistically significant.

Conversely, Huang Z *et al.* (2009)[17] found that EMMPRIN expression was significantly related to clinical stages with higher expression in stage III and IV cases of tongue OSCCs.

Thus, EMMPRIN expression adds a predictive power of the outcome of pathological stages.[18]

EMMPRIN expression was assessed in different histological grades of OSCC according to Bryne's grading system. We found that EMMPRIN expression was strong in all high-grade tumors, i.e., 12 cases (100%) and 5 cases (100%), respectively. Only 4 cases (30.77%) of well-differentiated squamous cell carcinoma cases showed strong expression. There was a statistically significant difference between EMMPRIN expression and histological grades of OSCC. ($P = 0.0021$) [Table 3 and Graph 2].

Similarly, Monteiro *et al.*[4] found EMMPRIN overexpression in high grades of OSCC with statistically significant results emphasizing the biological significance of this marker in tumor growth and progression of OSCC.

In contrast to our findings, Huang Z *et al.* (2009)[17] found statistically significant results in which moderately differentiated squamous cell carcinomas of the tongue showed higher EMMPRIN expression.

Arora *et al.* (2018)[12] in their study found intense EMMPRIN expression in oral epithelial dysplasia. EMMPRIN expression in OSCC cases was intense in well-differentiated OSCC cases, moderate staining in moderately differentiated and poorly differentiated OSCC cases.

In our study, EMMPRIN expression increased with histological grades of OSCC [Figures 1-3]. This upregulated EMMPRIN expression might contribute to tumorigenesis, growth, angiogenesis and local invasion. It could be thus considered as an objective and effective marker to predict the invasion and prognosis of OSCC.

In the present study, EMMPRIN was overexpressed in all cases of OSCC and showed strong expression in advanced stages and high-grade tumors, suggesting that it might be involved in the progression of these tumors. EMMPRIN overexpression indicates that this protein could be used as an important biological prognostic marker to identify high-risk OSCC patients. Furthermore, the high expression of this receptor could be regarded as an excellent potential therapeutic target against OSCC.[4]

The limited sample size, lack of uniform distribution of cases in stages and grades of OSCC, lack of direct methods in assessing EMMPRIN and unspecified survival rate of the patients may be the reasons for differences between our results and previous studies.

Taking into consideration all the studies performed on EMMPRIN expression independently and discrepancies found in them, it is more encouraging to investigate these markers with larger sample sizes, longer follow-up and more advanced methods.

CONCLUSION

On the assessment of EMMPRIN expression in OSCC, EMMPRIN expression increased in advanced clinical stages and higher histological grades of OSCC, suggesting that it might be involved in the progression and spread of these tumors. Thus, EMMPRIN can be used as an effective

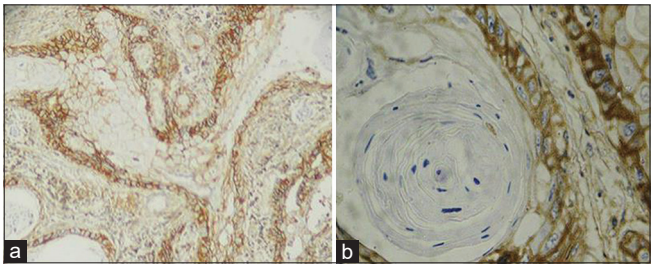


Figure 1: (a and b) Photomicrograph of well differentiated squamous cell carcinoma showing weak extracellular matrix metalloproteinase inducer expression under ×100 and ×400 respectively

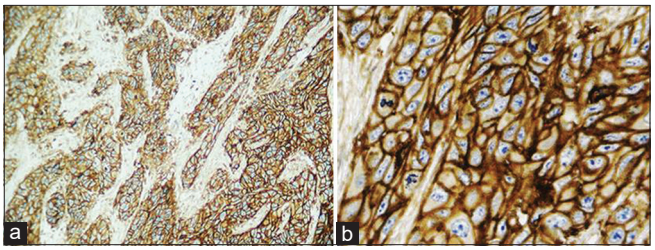


Figure 2: (a and b) Photomicrograph of moderately differentiated squamous cell carcinoma showing strong extracellular matrix metalloproteinase inducer expression under ×100 and ×400 respectively

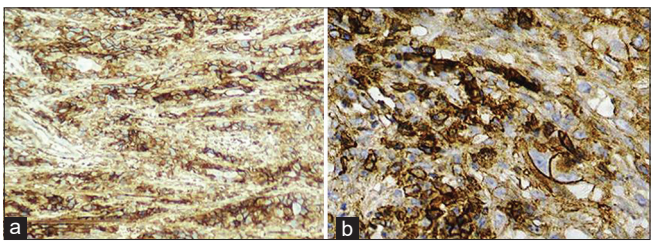


Figure 3: (a and b) Photomicrograph of poorly differentiated squamous cell carcinoma showing strong extracellular matrix metalloproteinase inducer expression under ×100 and ×400 respectively

biological prognostic marker to identify high-risk OSCC patients.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Fujii N, Shomori K, Shiomi T, Nakabayashi M, Takeda C, Ryoke K, *et al*. Cancer-associated fibroblasts and CD163-positive macrophages in oral squamous cell carcinoma: Their clinicopathological and prognostic significance. J Oral Pathol Med 2012;41:444-51.
2. Tang X, Mo C, Wang Y, Wei D, Xiao H. Anti-tumour strategies aiming to target tumour-associated macrophages. Immunology 2013;138:93-104.
3. Patel S, Shah K, Mirza S, Daga A, Rawal R. Epigenetic regulators governing cancer stem cells and epithelial-mesenchymal transition in oral squamous cell carcinoma. Curr Stem Cell Res Ther 2015;10:140-52.
4. Monteiro LS, Delgado ML, Ricardo S, Garcez F, do Amaral B, Pacheco JJ, *et al*. EMMPRIN expression in oral squamous cell carcinomas: Correlation with tumor proliferation and patient survival. Biomed Res Int 2014;2014:905680.
5. Chin D, Boyle GM, Williams RM, Ferguson K, Pandeya N, Pedley J, *et al*. Novel markers for poor prognosis in head and neck cancer. Int J Cancer 2005;113:789-97.
6. Daniel FI, Fava M, Hoffmann RR, Campos MM, Yurgel LS. Main molecular markers of oral squamous cell carcinoma. Appl Cancer Res 2010;30:279-88.
7. Xiong L, Edwards CK 3rd, Zhou L. The biological function and clinical utilization of CD147 in human diseases: A review of the current scientific literature. Int J Mol Sci 2014;15:17411-41.
8. Sweeny L, Dean NR, Frederick JW, Magnuson JS, Carroll WR, Desmond RA, *et al*. CD147 expression in advanced cutaneous squamous cell carcinoma. J Cutan Pathol 2012;39:603-9.
9. Guo H, Li R, Zucker S, Toole BP. EMMPRIN (CD147), an inducer of matrix metalloproteinase synthesis, also binds interstitial collagenase to the tumor cell surface. Cancer Res 2000;60:888-91.
10. Greene FL, Page DL, Fleming ID, Fritz AG, Balch CM, Haller DG, *et al*. AJCC Cancer Staging Manual. 6th ed.. Chicago (USA): Springer; 2002.
11. Kefeli M, Taslak Sengul A, Yildiz L, Baris S, Basoglu A, Kandemir B, *et al*. EMMPRIN and fascin expression in non-small cell lung carcinoma. Cent Eur J Med 2010;5:659-65.
12. Arora M, Mane DR. Immunohistochemical expression of extracellular matrix metalloproteinase inducer (EMMPRIN) in normal oral mucosa, oral epithelial dysplasia and oral squamous cell carcinoma. J Oral Maxillofac Pathol 2018;22:279-80.
13. Huang Z, Tan N, Guo W, Wang L, Li H, Zhang T, *et al*. Overexpression of EMMPRIN isoform 2 is associated with head and neck cancer metastasis. PLoS One 2014;9:e91596.
14. Igakura T, Kadomatsu K, Taguchi O, Muramatsu H, Kaname T, Miyauchi T, *et al*. Roles of basigin, a member of the immunoglobulin superfamily, in behavior as to an irritating odor, lymphocyte response and blood – Brain barrier. Biochem Biophys Res Commun 1996;224:33-6.
15. Jadhav KB, Gupta N. Clinicopathological prognostic implicators of oral squamous cell carcinoma: Need to understand and revise. N Am J Med Sci 2013;5:671-9.
16. Nair S, Singh B, Pawar PV, Datta S, Nair D, Kane S, *et al*. Squamous cell carcinoma of tongue and buccal mucosa: Clinico-pathologically different entities. Eur Arch Otorhinolaryngol 2016;273:3921-8.
17. Huang Z, Huang H, Li H, Chen W, Pan C. EMMPRIN expression in tongue squamous cell carcinoma. J Oral Pathol Med 2009;38:518-23.
18. El-Rehim DM, El-Maqsoud NM, El-Hamid AM, El-Bab TK, Galal EM. Expression of extracellular matrix metalloproteinase inducer and fascin in urinary bladder cancer: Correlation with clinicopathological characteristics. Mol Clin Oncol 2013;1:297-304.