Extracellular Matrix Metalloproteinase Inducer (EMMPRIN/CD147) Expression and its Correlation with Progression of Oral Squamous Cell Carcinoma

* Saha MK,[1] Quruni MO,[2] Afrin SS, [3] Hossain MS,[4] Mahmud F,[5] Mahmud SA,[6] Jinnah MA,[7] Dewan MR[7]

Abstract

Background: Oral cancer remains a major public health problem all over the world. Most of them are squamous cell carcinoma. It is most common in fifth decade of life. The aim of this study was to assess the distribution and pattern of EMMPRIN (CD147) expression and to assess the significance of its expression with clinicopathological parameters in relation to cancer progression.

Methods: This descriptive cross sectional study was carried out at the Department of Pathology, Dhaka Medical College over a period of two years from January 2017 to December 2018. A total 50 patients of different sex and age groups with oral squamous cell carcinoma were enrolled in this study. During the collection of specimen, all relevant information's were recorded systematically in a prepared proforma. All obtained specimens were immersed in 10% buffered formalin. Then proper H&E and immunostaining were done according to the standard protocol. H&E stained slides were categorized as different variety of squamous cell carcinoma. EMMPRIN staining was evaluated on the basis of extent and intensity of immune-labeling tumor cells.

Results: Patients' ages ranged from 20 to 80 years and distribution of sex were more common in female (56%) than male (44%). OSCC was highest on the buccal mucosa (50%), followed by tongue (12.0%), hard palate (12%) & Gingiva (10.0%) then other sites. Most common clinical presentation was ulcerated (52%), followed by swelling/lump (26%), exophytic (20%) and white patch (2%). In this study Grade I (48%) was the most prevalent grade followed by grade II (38%) and grade III (14%). In grade-I, 37.5% were with EMMPRIN high/over expression; in grade II, 89.5% presented with high/over expression and in grade III, all the patients were with high/over expression. There was statistically significant ($p<0.05$) difference observed between increasing grade and level of expression. EMMPRIN over expression was in stage-I (24.2%), stage-II (27.3%), stage-III (15.2) and stage-IV (33.3%). On the other hand, low expression was observed mostly in stage-I (70.6%) then stage-II (17.6%), stage III & stage IV (5.9%) each. Statistically significant ($p<0.05$) difference were observed among advanced stages and level of expression.

Conclusions: EMMPRIN (CD147) protein is widely expressed in all the cases of OSCC in our population with a significant association with grading and staging in relation to cancer progression. So it could be considered as an objective and effective marker to predict the invasion and prognosis of OSCC and could be an appropriate target for immunotherapeutic approaches.

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Keywords: EMMPRIN: Extracellular Matrix Metalloproteinase Inducer, OSCC: Oral squamous cell carcinoma, VC: Verrucous carcinoma, MMP: Matrix metalloproteinase, EMT: Epithelial to mesenchymal transition.

1. **Dr. Mridul Kumar Saha, Assist Professor, Department of Pathology, Colonel Malek Medical College, Manikganj. mridulkumarsaha@yahoo.com*
2. *Dr. Md. Oyes Quruni, Assistant Professor, Department of Pathology, Sheikh Hasina Medical College, Tangail.*
3. *Dr. Syeda Sadia Afrin, Lecturer, Department of Pathology, Dhaka Medical College.*
4. *Dr. Md. Shahadat Hossain, Lecturer, Lecturer, Department of Pathology, Dhaka Medical College.*
5. *Dr. Farhad Mahmud, Associate Professor, Department of Pathology, Colonel Malek Medical College, Manikganj.*
6. *Dr. SM A Mahmud, Lecturer, Department of Pathology, Dhaka Medical College.*
7. *Dr. Mohammad Shahed Ali Jinnah, Associate Professor, Dhaka Medical College.*
8. *Professor Dr. Md. Rezaul Karim Dewan, Head of the Department, Department of Pathology, Dhaka Medical College.*

**For correspondence*

Introduction

Head and neck cancer is the sixth most common cancer. In 48% of cases, the tumors are located in the oral cavity and 90% of them are squamous cell carcinomas.[1] Oral squamous cell carcinoma (OSCC) ranks among the top ten most frequently cancers with bad prognosis.[2] Little is known about the molecular events that govern OSCC initiation, progression and metastasis.[3]

Extracellular matrix metalloproteinase inducer (EMMPRIN) is known as CD147, Basignin, M6, Neurothelin, or gp42 which is present in squamous epithelial cells. It also presents in other epithelial cells, neuronal or nerve cells, myocardial cells, lymphoid cells and germ cells. [4] It is expressed in several cancers including head and neck squamous-cell carcinomas, pancreatic adenocarcinomas, kidney chromophobic carcinomas, hepatocellular carcinomas, medullary breast adenocarcinoma, cervical carcinomas and glioblastoma. [4]

EMMPRIN contributes to cell adhesion modulation, tumor growth, invasion, metastasis and angiogenesis by association with different signaling pathways.[5] It plays a vital role in survival of malignant cells also.[6] EMMPRIN has been identified as a tumor-cell membrane protein that stimulates MMP (Matrix metalloproteinase) production in stromal fibroblasts. This extracellular matrix metalloproteinase contribute to epithelial to mesenchymal transition (EMT) to promote tumor invasion and lymph node metastasis.[7] Recently, it has been reported to be a useful diagnostic and prognostic marker as it is involved in various aspects of cancer progression. This can help to treat a large number of population with oral squamous cell carcinoma. Previously no study was done in Bangladesh with this marker. The findings of the current study may highlight the significance of EMMPRIN (CD147) expression in relation to cancer progression thus can become an attractive target for immunotherapeutic approaches.

Methods

This was a descriptive cross-sectional study. This study was carried out at the Department of Pathology, Dhaka medical college over a period of two years from January 2017 to December 2018. A total 50 patients of different sex and age groups with oral squamous cell carcinoma were enrolled in this study. Patients of any age group with histopathologically diagnosed squamous cell carcinoma of the oral cavity were included in this study and patients who received neo-adjuvant or adjuvant chemotherapy due to carcinoma of oral cavity were excluded from this study. To do this study ethical clearance was taken from institutional ethical committee of Dhaka Medical College. During the collection of specimen, all relevant information's were recorded systematically in a prepared proforma. All obtained specimens were immersed in 10% buffered formalin. Proper H&E and immunostaining were done according to the standard protocol. H&E stained slides were categorized as different variety of squamous cell carcinoma. EMMPRIN staining was evaluated on the basis of extent and intensity of immune-labeling tumor cells. EMMPRIN showed cytoplasmic and membranous staining patterns for the positive cells. All the cases were numbered chronologically and the same number was given to histological as well as in immunohistochemically stained slides. This result is in accordance with studies done by Piao et al. (2012) and Yang et al. (2013)[4,8] who examined its immunohistochemistry in hypopharyngeal carcinoma and salivary duct carcinoma respectively. The intensity of staining was scored as 0(absent), 1 (weak), 2 (moderate), and 3 (strong). The extent of membrane tumor cells staining was semi-quantitatively evaluated as 0 (no labelling or

labelling in <10% of tumor cells), 1 (labelling in 10% to 24% of tumor cells), 2 (labelling in 25% to 49% of tumor cells), 3 (labelling in 50% to 74% of tumor cells) and 4 (labelling in 75% or more of tumor cells).

The sum of the intensity and extent scores were used as the final score (0–7). Tissues having a final score of 0-1 were considered negatives. Final scores of 2-3, 4-5, and 6-7 were considered 1+, 2+, and 3+, respectively. For data analysis, score 3+ was defined as EMMPRIN overexpression. All necessary and relevant data were processes. Data were evaluated by standard statistical methods. Analysis was done by SPSS (Statistical Package for Social Science) by applying appropriate formula.

Results

Table I: Shows age distribution of the patients. The mean (± SD) of the total number of patients was 56.74 (± 11.90) years with range from 20 to 80 years and maximum (58.0%) number of cases were between 41 to 60 age group

Age (Years)	Frequency	Percent
20-40	4	8.0
41-60	29	58.0
61-80	17	34.0
Total	50	100.0

Table II: OSCC was highest on the buccal mucosa (50%) followed by tongue (12.0%), hard palate (12%) & Gingiva (10.0%) then other sites

Site of lesion	Histologic Subtype					Total
	Conventional	Verrucous	Spindle cell type	Papillary	Basaloid	
Buccal mucosa	23 (46.0)	2 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	25 (50.0)
Tongue	6 (12.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (12.0)
Gingiva	5 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (10.0)
Hard palate	4 (8.0)	0 (0.0)	1 (2.0)	0 (0.0)	1 (2.0)	6 (12.0)
Lip	4 (8.0)	0 (0.0)	0 (0.0)	1 (2.0)	0 (0.0)	5 (10.0)
Floor of mouth	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.0)
Angle of mouth	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.0)
Retromolar area	0 (0.0)	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.0)
Total	44 (88.0)	3 (6.0)	1 (2.0)	1 (2.0)	1 (2.0)	50 (100.0)

Figure within parenthesis indicates percentage.

Table III: Illustrates grade I (48%) was the most prevalent grade in this study followed by grade II (38%) & grade III (14%)

Grade	Histologic Subtype					Total
	Conventional	Verrucous	Spindle cell type	Papillary	Basaloid	
Grade I	21 (47.7)	3 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	24 (48.0)
Grade II	18 (40.9)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	19 (38.0)
Grade III	5 (11.4)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)	7 (14.0)
Total	44 (100.0)	3 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	50 (100.0)

Table IV: The most common predominant buccal mucosa showed 48.5% with high/over expression and 52.9% low expression. There was no statistical significance (p>0.05) observed between site of lesion and level of EMMPRIN expression

Site of lesion	Expression		Total n (%)	p value
	Low n (%)	High/Over n (%)		
Buccal mucosa	9 (52.9)	16 (48.5)	25 (50.0)	
Tongue	1 (5.9)	5 (15.2)	6 (12.0)	
Gingiva	2 (11.8)	3 (9.1)	5 (10.0)	
Hard palate	0 (0.0)	6 (18.2)	6 (12.0)	0.111[a]
Lip	4 (23.5)	1 (3.0)	5 (10.0)	
Floor of mouth	0 (0.0)	1 (3.0)	1 (2.0)	
Angle of mouth	0 (0.0)	1 (3.0)	1 (2.0)	
Retromolar area	1 (5.9)	0 (0.0)	1 (2.0)	
Total	17 (100.0)	33 (100.0)	50 (100.0)	

[a]Chi-Square test was done to measure the level of significance.

Table V: Distribution of the patients with SCC according to clinical presentation by EMMPRIN expression. The predominant clinical presentation was ulcerated lesion which showed maximum (69.7%) high/over expression followed by swelling/lump (24.2%) & exophytic (6.1%).

	Expression			
Clinical presentation	Low n (%)	High/Over n (%)	Total n (%)	p value
Ulcerated	3 (17.6)	23 (69.7)	26 (52.0)	
Swelling/lump	5 (29.4)	8 (24.2)	13 (26.0)	0.001[a]
Exophytic	8 (47.1)	2 (6.1)	10 (20.0)	
White patch	1 (5.9)	0 (0.0)	1 (2.0)	
Total	17 (100.0)	33 (100.0)	50 (100.0)	

[a]Chi-square test was done to measure the level of significance.

Table VI: In low expression, most of the histologic subtype were conventional (76.5%), followed by verrucous (17.6%) and papillary (5.9%)

Histologic subtype	Expression		Total n (%)	p value
	Low n (%)	High/Over n (%)		
Conventional	13 (76.5)	31 (93.9)	44 (88.0)	
Verrucous	3 (17.6)	0 (0.0)	3 (6.0)	
Spindle cell type	0 (0.0)	1 (3.0)	1 (2.0)	0.057
Papillary	1 (5.9)	0 (0.0)	1 (2.0)	
Basaloid	0 (0.0)	1 (3.0)	1 (2.0)	
Total	17 (100.0)	33 (100.0)	50 (100.0)	

Table VII: Shows in grade I, 62.5% were low expression & 37.5% high/over expression. In grade II, maximum 89.5% presented with high/over expression & only (10.5%) low expression. On the other hands, in grade III, all patients were with high/over expression. There was statistically significant ($p<0.05$) difference observed between grade and level of expression.

Grade	Expression		Total n (%)	p value
	Low n (%)	High/Over n (%)		
Grade I	15 (62.5)	9 (37.5)	24 (100.0)	
Grade II	2 (10.5)	17 (89.5)	19 (100.0)	<0.001[a]
Grade III	0 (0.0)	7 (100.0)	7 (100.0)	
Total	17 (34.0)	33 (66.0)	50 (100.0)	

[a]Chi-Square test was done to measure the level of significance.

Table VIII: Shows among low expression, most of the cases shows stage-I (70.6%), stage-II (17.6%), stage III & stage IV were (5.9%) each. On the other hands, in high/over expression, stage I were24.2% stage-II (27.3%), stage-III (15.2) and stage-IV (33.3%). Statistically significant ($p<0.05$) difference were observed between stage and level of expression.

Staging	Expression		Total n (%)	p value
	Low n (%)	High/Over n (%)		
Stage I	12 (70.6)	8 (24.2)	20 (40.0)	
Stage II	3 (17.6)	9 (27.3)	12 (24.0)	0.013[a]
Stage III	1 (5.9)	5 (15.2)	6 (12.0)	
Stage IV	1 (5.9)	11 (33.3)	12 (24.0)	
Total	17 (100.0)	33 (100.0)	50 (100.0)	

[a]Chi-Square test was done to measure the level of significance. 56.74 ± 11.90 (20-80)

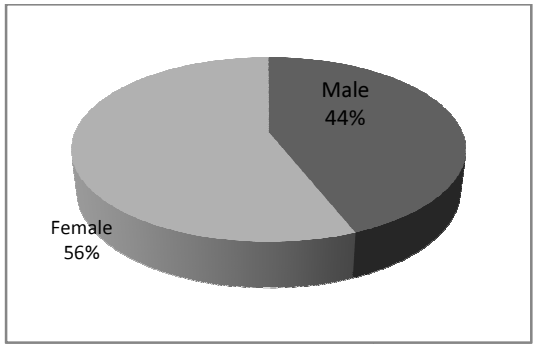


Figure 1. Pie chart of patient with SCC according to sex. The male & female ratio was 1: 1.27.

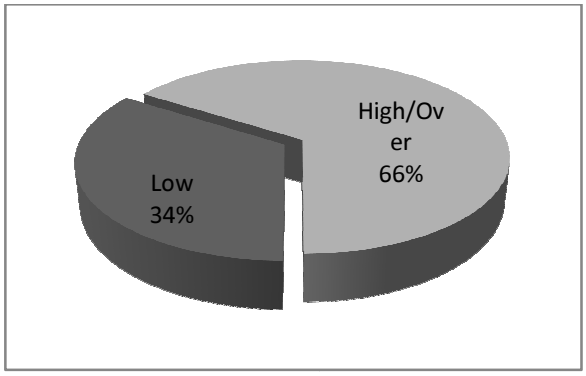
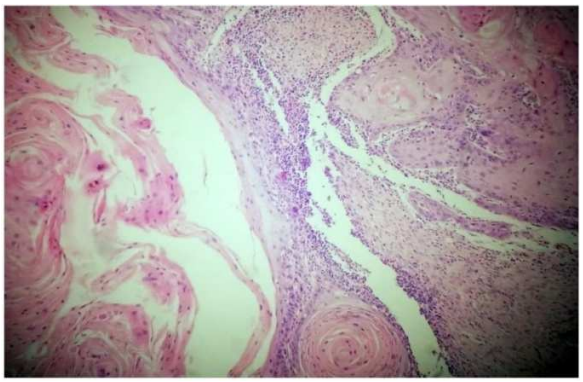
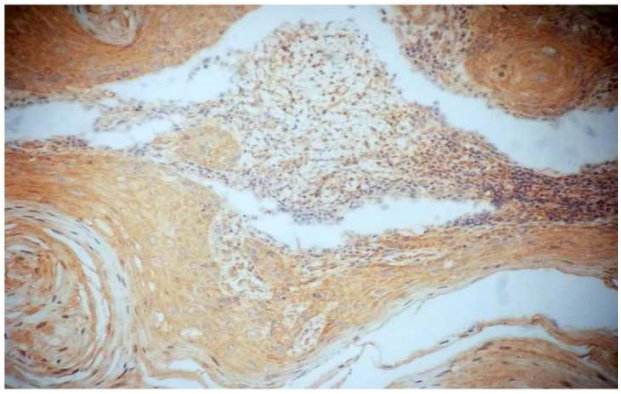


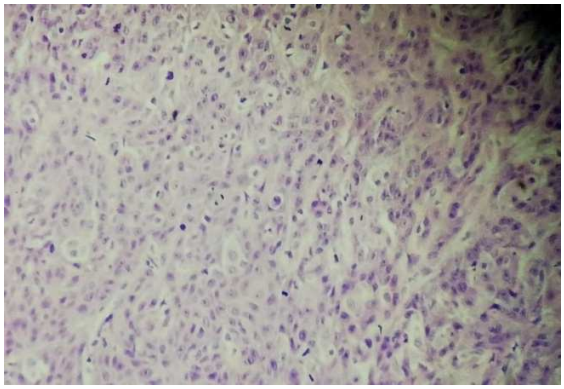
Figure 2. Pie chart of the patients with SCC according to expression



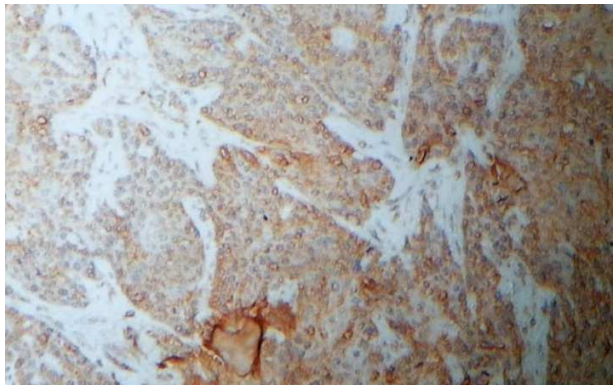
A. Photomicrograph showing a well differentiated squamous cell carcinoma. (Case no. 7; H&E X 400x)



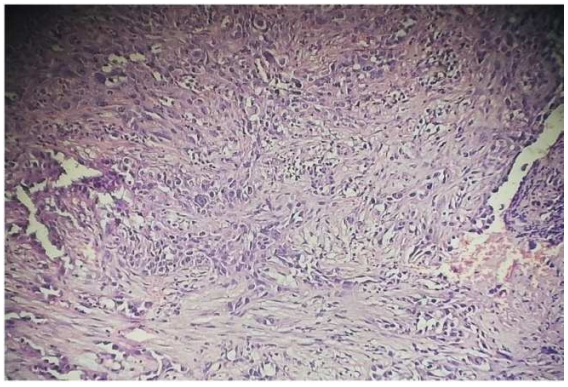
B. Photomicrograph showing high expression of CD 147 molecule with strong intensity in well differentiated squamous cell carcinoma (Case no. 7; EMMPRIN immunostain X 400x)



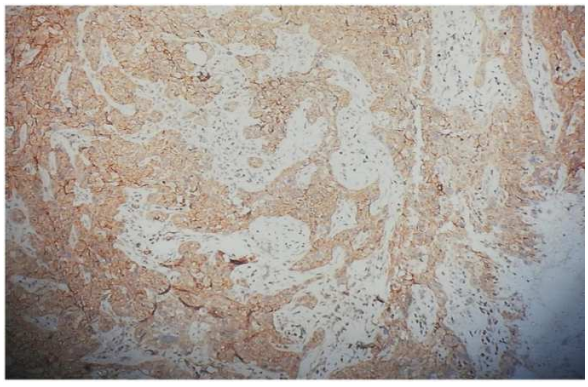
C. Photomicrograph showing a moderately differentiated squamous cell carcinoma. (Case no. 16; H&E X 400x)



D. Photomicrograph showing diffuse cytoplasmic and membrane positivity of EMMPRIN immunostain in more than 75% cells with moderate intensity. (Case no. 16; EMMPRIN immunostain X 400x)



E. Photomicrograph showing a poorly differentiated squamous cell carcinoma. (Case no. 47; H&E X 400x)



F. Photomicrograph showing diffuse cytoplasmic and membrane positivity of EMMPRIN immunostain in more than 75% cells. (Case no. 47; EMMPRIN immunostain X 400x)

Figure 3. Photomicrographs of H and E and immunostains.

Discussion

Oral carcinogenesis is a multistep phenomenon whose progression is segregated into the early and late stages. A series of molecular events bring about this progression from normal to dysplasia to carcinoma in situ to invasion and eventual metastasis. This progression is associated with controlled proteolysis, and involves interactions between the tumor cells and the extracellular matrix.[9] Being an important factor in epithelial and connective tissue interaction, EMMPRIN might be involved in OSCC progression and metastasis.

The mean age of occurrence of our study is (56.74 ± 11.90). Comparable age distribution is found in the study conducted by Rahman et al. (2014)[10] which showed most cases in the fifth decade of life and the mean age was 50.4±3. The gender related findings of the current study (Male: Female ratio, 1:1.27) are also consistent with the local study by Suporna Salea, (2017)[11] which showed the ratio of malignant lesion 1:1.23. This may be due to more tendency of betel nut chewing in female of our country.

The current study shows buccal mucosa (50%) was the most prevalent site followed by tongue and gingiva. The study conducted by Krishna et al.(2014) also identified buccal mucosa (35.5%) was the most prevalent site.[12] In this study, non-healing ulcer (52%) was the commonest presenting complain then swelling/lump, exophytic and white patch respectively.

Conventional variety of OSCC was the most prevalent subtype encountered in the current study (88%). The papillary OSCC was diagnosed in 2% of the present cases, while Thompson et al. (2003)[13] revealed that it constitutes approximately 1% of all OSCCs. Most studies have demonstrated that papillary OSCC shows male predilection and presents clinically as an exophytic mass[14] a finding corroborated by the present results. Verrucous carcinoma (VC) accounted for 6% of the total studied cases of the present study. Neville (2016) reported that VC accounts for 1-10% of all oral squamous cell carcinomas, depending on the local acceptance of smokeless tobacco use.[15] In this study, VC clinically presented at around an age of 60 years as an exophytic mass mostly on the buccal mucosa, which is in accordance with the reviewed literature and showed 4.5% of the total OSCC in head and neck region.

As for the histological grade, among the 50 OSCC cases investigated, well differentiated carcinomas made most of the tumor burden (48%). This result is consistent with Krishna et al. (2014),[12] Rahman et al. (2014)[10] showed well differentiated SCC was 39.9%, 42.8% and 70.73% respectively. Among 44 cases of conventional OSCC, most of the cases were well differentiated (47.7%) followed by moderate (40.9%) and poorly differentiated (11.4%) cases.

According to the immunostaining results of the present study, more than half of the OSCC cases (66%) were categorized as having high EMMRIN expression score and 34% cases showed low EMMPRIN expression scores. No case was detected as negative staining. Similar to these findings, Monteiro et al. (2014)[6] showed positive EMMPRIN (CD147) immunoreactivity in all examined OSCC cases with high expression in 75.7% of the cases. This suggests that EMMPRIN play an important role in oral tumor progression.

We found significant association (p=0.001) between the level of EMMPRIN expression with histological grades of OSCC. Monteiro et al. (2014)[6] and Ahmed et al. (2014)[16] demonstrated significant association of

EMMPRIN expression with histological grades of OSCC (p=0.002 & 0.005 respectively). This finding is consistent with our study. Ahamed et al. (2014)[16] showed over expression of EMMPRIN protein in well differentiated carcinoma 46.66%, in moderately 90% and 100% in poorly differentiated oral squamous cell carcinoma. Whereas in our study EMMPRIN over expression in poorly differentiated OSCCs 100%, moderate 89%, and well differentiated tumors 37.5%. This result represents that level of EMMPRIN expression increases with increased grading.

In this study we observed that EMMPRIN over expression was significantly associated with advanced clinical stages. In this current study EMMPRIN over expression was in stage-I, 24.2%; stage-II, 27.3%; stage-III, 15.2%; stage-IV, 33.3% and showed a significant p-value (0.013). This result correlates with Ahmed et al.(2014)[16] who reported as stage-I, 7.7%; stage-II, 34.6%; stage-III, 11.5% and stage-IV, 46.2% with also a significant p-value (0.047). In advanced clinical staging, EMMPRIN over expression was also increased. EMMPRIN plays a crucial role in tumor progression, invasion and metastasis in head and neck squamous cell carcinoma. So, it may represent a useful biomarker for prognostic evaluation. Furthermore, the measurement of EMMPRIN expression levels can assist in predicting patients' prognosis.

Conclusion

Highest number of cases was in fifth decade of life and the mean age of occurrence (56.74 ± 11.90). The gender related findings of the current study (Male: Female ratio, 1:1.27) showing female predominance. The buccal mucosa was the most frequently affected site (50%) and conventional OSCC was the most prevalent subtype. Among the 50 OSCC cases investigated, well differentiated carcinomas made most of the tumour burden (48%). More than half of the OSCC cases (66%) were categorized as having high EMMRIN expression score. Significant association (P=0.001) between EMMPRIN expression scores with histological grades and clinical staging of OSCC were found. Moderate (89%) and poorly differentiated (100%) OSCCs exhibited high expression score as compared to well differentiated tumors (37.5%). EMMPRIN (CD147) could be considered as an objective and effective marker to predict the invasion and prognosis of OSCC. This could be an appropriate target for immunotherapeutic approaches.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J and Thun MJ. Cancer statistics, 2009. CA: a cancer Journal for Clinicians. 2009; 59(4):225-249.
2. Specenier P, Vermorken JB. Advances in the systemic treatment of head and neck cancers. Current Opinion in Oncology. 2010 May 1; 22(3):200-5.
3. Siqueira AS, Gama-de-Souza LN, Arnaud MV, Pinheiro JJ, Jaeger RG. Laminin-derived peptide AG73 regulates migration, invasion, and protease activity of human oral squamous cell carcinoma cells through syndecan-1 and β1 integrin. Tumor Biology. 2010 Jan 1; 31(1):46-58.
4. Yang Q, Liu Y, Huang Y, Huang D, Li Y, Wu J, Duan M. Expression of COX-2, CD44v6 and CD147 and relationship with invasion and lymph node metastasis in hypopharyngeal squamous cell carcinoma. PLoS One. 2013 Sep 3; 8(9): e71048.
5. Iacono KT, Brown AL, Greene MI, Saouaf SJ. CD147 immunoglobulin superfamily receptor function and role in pathology. Experimental and molecular pathology. 2007 Dec 1; 83(3):283-95.
6. Monteiro LS, Delgado ML, Ricardo S, Garcez F, Amaral BD, Pacheco JJ, Lopes C, Bousbaa H. EMMPRIN expression in

oral squamous cell carcinomas: correlation with tumor proliferation and patient survival. BioMed research international. 2014 Jan 1; pp 905-680.

7. Siu A, Chang J, Lee C, Lee S and Ramos DM. Expression of EMMPRIN modulates mediators of tumor invasion in oral squamous cell carcinoma. Journal of the California Dental Association. 2013; 41(11):831-838.
8. Piao S, Zhao S, Guo F, Xue J, Yao G, Wei Z, Huang Q, Sun Y, Zhang B. Increased expression of CD147 and MMP-9 is correlated with poor prognosis of salivary duct carcinoma. Journal of cancer research and clinical oncology. 2012 Apr 1; 138(4):627-35.
9. Shrestha B, Subedi S, Bayracharya D, Radhakrisnan R. Immunohistochemical expression of MMP-2 in an experimental progression model of oral cancer. Journal of Chitwan Medical College. 2014 Jul 30; 4(2):3-8.
10. Biswas R, Rabbani R, Ahmed HS, Sarker MA, Zafrin N, Rahman MM. Antibiotic sensitivity pattern of urinary tract infection at a tertiary care hospital. Bangladesh Critical Care Journal. 2014 Aug 11; 2(1):21-4.
11. Saleh S. Histomorphological pattern of oral and oropharyngeal lesion: Study in a tertiary care hospital 2017 Dec 31;(Thesis) pp 81-82.
12. Krishna A, Singh RK, Singh S, Verma P, Pal US, Tiwari S. Demographic risk factors, affected anatomical sites and clinicopathological profile for oral squamous cell carcinoma in a north Indian population. Asian Pac J Cancer Prev. 2014 Jan 1; 15(16):6755-0.
13. Thompson LD. Squamous cell carcinoma variants of the head and neck. Current Diagnostic Pathology. 2003 Dec 1; 9(6):384-96.
14. Cardesa, A., 2005. Squamous cell carcinoma. World Health Organization Classification of Tumours. Pathology and Genetic of Head and Neck Tumours, pp.118-119.
15. Neville BW, Damm DD, Allen CM, Chi AC. Oral and maxillofacial pathology. Elsevier Health Sciences; 2015 May 13, pp.550-554.
16. Ahmed EM, Frag AS. Expression of EMMPRIN/CD147 and Ki-67 in oral squamous cell carcinoma: an immunohistochemical study. J Am Sci. 2014; 10:241-9.