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Correlation of E-cadherin Immunoexpression with Histopathological Grading of Oral Squamous Cell Carcinoma

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Abstract

Background: Cancers arising from the oral cavity are mostly squamous cell carcinoma. E-cadherin is encoded by the CDH1 gene that helps in maintaining cell polarity and normal tissue architecture. Loss of E-cadherin expression is associated with tumor differentiation, invasion, metastasis and poor prognosis. **Objectives:** To assess the E-cadherin expression in different grades of oral squamous cell carcinoma (OSCC) and to determine the association of E-cadherin expression with histological grades, age & gender of OSCC patients. **Methods:** This cross-sectional observational study was conducted at the Department of Pathology, Sylhet MAG Osmani Medical College, from July 2021 to June 2022. A total of 53 (small biopsy and resected) OSCC cases were processed; paraffin blocks were made and stained with routine H&E stain. The sections were examined microscopically and the tumors were graded histologically. Immunohistochemistry was performed by using a commercially available anti-E-cadherin antibody. Immunoreactivity was calculated by multiplying the expression score and the intensity score. **Results:** Out of 53 cases of OSCC, most of the cases (35/53) were found in the buccal mucosa (66.0%) followed by in the tongue (18.9%). 50.9% of patients belonged to Grade-I, 43.4% were Grade-II and 5.7% were Grade-III OSCC. Regarding E-cadherin, 56.6% of cases showed positive expression, 39.6% cases showed reduced expression and 3.8% cases showed negative expression. Negative expression was found only in 2 (66.7%) Grade III cases of OSCC. A significant correlation was seen between E-cadherin expression with histological grading but not with age and gender. **Conclusion:** E-cadherin might be a valuable tool for predicting OSCC patient outcomes and it can be further used for therapeutic targets.

Keywords: OSCC, E-cadherin, Immunoexpression

Introduction

The oral cavity represents the upper part of the alimentary tract. It begins from the lips and cheeks outwardly to the anterior pillar of the fauces on the inside, where it connects with the oropharynx. The roof of the mouth is formed by the palate and the floor of the mouth is designed mainly by the tongue. The lateral walls of the mouth are demarcated by the cheeks and retromolar regions.[1]

Oral malignancy commonly concerns the area of the lips, tongue, mouth and oropharynx. Oral squamous cell carcinoma comprises 90% of all Head-Neck squamous cell carcinomas.[2] It is one of the leading malignancies in developing countries though it was less dominant in developed western countries. But nowadays the trend has changed due to modifications in lifestyle, Bangladesh, India, Pakistan and Sri Lanka calculate nearly one-fourth of all new cases of oral carcinomas.[3]

The GLOBOCAN (project of IARC, WHO) estimates that in 2020, worldwide newly diagnosed oral cavity & lip cancers 3,77,713 (2% of all malignancies) and 1,77,757 (1.8% of all malignancies) cases died; In the year 2022, newly diagnosed oral cavity & lip cancers are 13,985 (8.9% of all malignancies) and 8,137 (7.9% of all malignancies) cases have died in Bangladesh.

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The prevalence of oral squamous cell carcinoma is 3.55% in Bangladesh.[4]

As per the record book of the pathology department (Sylhet MAG Osmani Medical College) in 2020 & 2021 total number of diagnosed oral squamous cell carcinoma was 31 & 37 respectively.

The use of tobacco and the areca nut (betel) yields potentially malignant injuries from which oral cancer may develop particularly in developing countries. Consumption of alcohol is also important in oral carcinogenesis, particularly in developed countries. Other predisposing factors of OSCC include dietary habits (decreased consumption of fresh vegetables and increased consumption of meat), immunodeficiency, human papillomavirus (HPV 16/18) infection and poor oral hygiene.[4]

E-cadherin is encoded by the CDH1 gene located on chromosome 16q21. It is a calcium-dependent trans-membrane glycoprotein that is expressed in most epithelial cells and helps in maintaining cell polarity and normal tissue architecture. The intracellular domain of E-cadherin is linked to the actin cytoskeleton through the catenin and catenin.[5]

The loss of the E-cadherin catenin complex leads to the reduction or absence of epithelial cadherin expression in the cell membranes, cytoplasmic accumulation of catenin and its translocation to the nucleus, contributing to carcinogenic events.[6] Disruption of the cadherin-catenin complex has been demonstrated in various cancers and has been correlated with tumor differentiation invasion metastasis and prognosis.[7]

Epithelial-mesenchymal transition (EMT) plays an important role in cancer progression, metastasis, and chemoresistance, most likely involving a common molecular mechanism. A hallmark of EMT is the down-regulation of the cell adhesion molecule E-Cadherin.[8]

Accordingly, low expression of E-cadherin in OSCC has been associated with clinical and histological features of malignancy, such as poor tumor differentiation, metastasis, recurrence and low survival.[9] Several studies suggest that the E-cadherin gene (CDH1) is a predictor of the prognosis of oral squamous cell carcinoma.[10]

Immunohistochemistry (IHC) is a globally accepted diagnostic tool that detects various gene expression at the protein level. E-cadherin protein expression detected by immunohistochemistry could be a valid prognostic marker in patients with various carcinomas.[11]

Few studies show that Histone Deacetylase Inhibitors (HDACI) are associated with suppression of EMT in various tumors. HDACI reverses EMT through the upregulation of E-cadherin in different solid tumors such as head & neck SCC, Hepatocellular carcinoma, Breast carcinoma and Esophageal carcinoma.[13] Vorinostat (under trial) is a promising HDACI in carcinoma of the oral cavity to up-regulate E-cadherin and reverse EMT.[11]

This study will be an important adjunct along with grading, to determine the prognosis and also to plan the treatment option that would lead to lesser morbidity and increased survival of patients with OSCC.

Materials and methods

This cross-sectional observational study was carried out in the Department of Pathology Sylhet MAG Osmani Medical College, Sylhet from July 2021 to June 2022. The study was approved by the Institutional Ethics Committee before its commencement. The sample size was 53 and the sampling technique was nonrandom convenience sampling. Among 53 cases, 41tissue samples were taken from the Department of Otolaryngology & Head-Neck Surgery and the Department of Oral & Maxillofacial Surgery, Sylhet MAG Osmani Medical College, and 12 paraffin blocks were collected from other private hospitals of Sylhet city. These samples were chronologically gross examination, tissue processing and staining, microscopic analysis, and immunohistochemical examination was done. Detailed clinical information was obtained by taking history and recorded in a data collection sheet. Filling up of the data sheet was performed in all cases either from the patient or patient's attendant's statement and the patient's clinical and investigation files. All the cases were numbered chronologically and the same number was given to histological as well as immunohistochemical slides.

Expression score

Expression score (ES)	% of cells stained
0	Negative
1	<10
2	11-50
3	51-80
4	>80

Tumour cells expression score (ES): [(0=negative), (1=<10% staining), (2=11%-50% staining), (3=51%-80% staining) and(4 =≥80% staining)]

Intensity score

Intensity score (IS)	Intensity of staining
0	Negative
1	Weak
2	Moderate
3	Strong

Intensity of staining: [(0=Negative), (1=weak), (2=moderate) and (3=strong)]

Total score

Total score (TS)= (TS=ES×IS)	Grading
0	Negative
1-4	Reduced
5-12	Positive

Total score: [(0=Negative), (1-4=Reduced), (5-12=Positive)]

Results

In this study, the sample size was 53. out of fifty-three (53) study cases, 30.2% of the patients were from the 51-60 years age group. The mean age of the patients was 55.9±14.5 years. 56.6% of patients belonged to females with a male-female ratio is 1:1.30. Most of the tumors were found in buccal mucosa (66.0%) followed by tongue (18.9%). Rest were in the retromolar area (7.5%), lip (5.7%), and angle of mouth (1.9%) [(Table 01)]. Histologically, 50.9% of patients belonged to Grade-I, 43.4% to Grade-II and 5.7% to Grade-III [(Table 02)].

It is observed that among fifty-three (53) study cases, 56.6% of cases showed positive expression, 39.6% cases showed reduced expression and 3.8% cases showed negative expression [(Table 03)]. Out of a total of 27 cases of Grade-I OSCC, E-cadherin expression was found positive in 66.7% of cases, reduced in 33.3% of cases. Among 23 cases of Grade-II OSCC, E-cadherin expression was found positive in 52.2% of cases, reduced in 47.8% of cases. Negative expression was not found in either Grade-I and Grade-II OSCC. Out of three (03) G-III OSCC, E-cadherin was reduced in 33.3% of cases, negative in 66.7% of cases and no positive expression was seen. The association between E-cadherin expression and histologic grades was done and the result was statistically significant (p=0.003) [(Table 04)].

Table 01: Distribution of the study cases according to the anatomic site of OSCC

Anatomic Site	Frequency	Percentage
Buccal mucosa	35	66.0
Retromolar area	04	7.5
Lip	03	5.7
Tongue	10	18.9
Angle of the mouth	01	1.9
Total	53	100.0

Table 02: Distribution of the study cases according to histologic grades of OSCC

Histologic grades of OSCC	Frequency	Percentage
Grade I	27	50.9
Grade II	23	43.4
Grade III	03	5.7
Total	53	100.0

Table 03: Distribution of the study cases according to E-cadherin immunoexpression

E-cadherin immunoexpression	Frequency	Percentage
Positive	30	56.6
Reduced	21	39.6
Negative	02	3.8
Total	53	100.0

Table 04: Association between E-cadherin expression and histologic grades of OSCC

Histological grades	E-cadherin expression			P value
	Positive	Reduced	Negative	
Grade-I	18 (66.7)	9 (33.3)	0 (0.0)	0.003[s]
Grade-II	12 (52.2)	11 (47.8)	0 (0.0)	
Grade-III	0 (0.0)	1 (33.3)	2 (66.7)	
Total	30 (56.6)	21 (39.6)	2 (3.8)	

*Fisher's Exact test was done to measure the level of significance.

Figures within parenthesis indicate the corresponding percentage.

's' indicates significant.

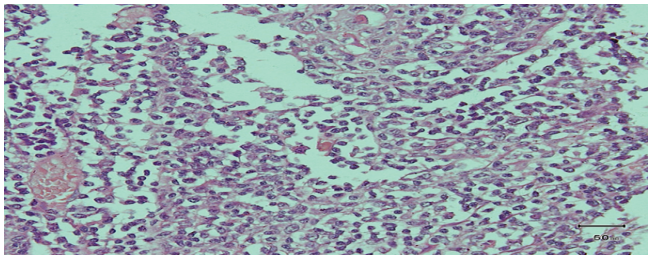

Figure 01: 11.8: Photomicrograph of histopathological section of moderately differentiated OSCC (Case no. 24; H&E stain, 400X magnification).

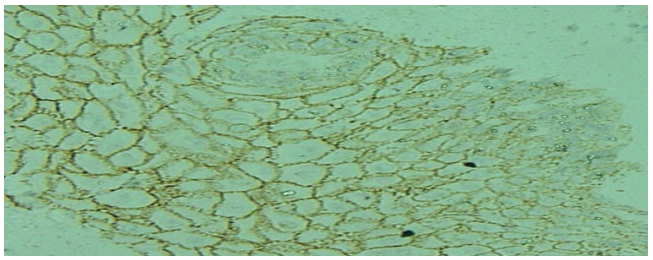

Figure 02: 11.10: Photomicrograph of a section showing E-cadherin in moderately differentiated OSCC positive expression<60%% (3)×(3)Intensity =9(Case no: 24; IHC, 400X magnification).

Discussion

Squamous cell carcinoma is the most common malignancy affecting the oral cavity. It continues to pose a serious threat to the oral health. Despite vast advancements in the field of cancer research, availability of sophisticated diagnostic techniques and improved therapeutic options, the prognosis of such patients remains very poor.[12]

Many studies revealed that the downregulation of E-cadherin in OSCC is associated with poor tumor differentiation, metastasis, recurrence and low survival rate.[8-10,13] So, E-cadherin expression would be a desirable prognostic factor that aids in proper treatment and overall survival of the patients.

In the current study, most of the OSCCs were arising from buccal mucosa 35 (66%) followed by tongue 10 (18.9%). Similar findings were reported by two articles.[4,5] However, in contrast to the findings of the current study reported that the tongue was the most common site of OSCC.[13-16] Alveolus and gingiva were the commonest sites of OSCC that were observed in two articles[17,18] respectively.

In the present study, out of 53 study cases, most of the cases were Grade-I (51%) OSCC followed by Grade-II (43%) and Grade-III (6%). Predominantly Grade-I OSCC was also observed in several articles[5,17,18] that were 50%, 46% and 59% respectively. Another two articles[15,19] reported that Grade-II OSCC were 50% and 37.5% respectively which were not in concordance with this study. Grade-III OSCC were 42.67% and 45.23% reported respectively in two articles[4,10]; which were not in concordance with this study. In this study, most of the cases were Grade-I OSCC. This may be due to the enrollment of small biopsy samples, small sample size, and not multicentered study.

Regarding E-cadherin expression, among 53 study cases, 30 (56.6%) cases showed positive expression, 21 (39.6%) cases showed reduced expression and 2 (3.8%) cases showed negative expression. Out of a total of 27 cases of Grade-I OSCC, E-cadherin expression was found positive in 18 (66.7%) cases, and reduced in 09 (33.3%) cases. Among 23 cases of Grade-II OSCC, E-cadherin expression was found positive in 12 (52.2%) cases, and reduced in 11 (47.8%) cases. Negative expression was not found in either Grade-I or Grade-II OSCC. Out of three (03) G-III OSCC, E-cadherin was reduced in 01 (33.3%) cases, negative in 02 (66.7%) cases and no positive expression was seen.

In the present study, it was observed that there is a significant correlation between E-cadherin expression and histologic grades of OSCC (p=0.003). This result was in accordance with many articles.[3,5,15,17,20-22] However, this result was discordant with several other studies.[13,18,23]

Conclusion

OSCC is the most common malignancy in the head-neck region. The contribution of E-cadherin in OSCC is significant according to the present study. Most of the studies revealed that E-cadherin is a valuable prognostic marker for disease-free survival in OSCC patients. Some studies showed that Histone Deacetylase Inhibitors (HDACI) reverse EMT through the upregulation of E-cadherin. Therefore, E-cadherin might be a useful therapeutic target for OSCC in the near future.

References

1. Berkovitz, B.K. (2016) 'Oral cavity' in Standring, S., Anand, N., Birch, R., Collins, P., Crossman, A.R., Gleeson, M., Jawaheer, G., Smith, A.L., Spratt, J.D., Stringer, M.D., Tubbs, R.S., Tunstall, R., Wein, A.J. and Wigley, C.B. (eds) Gray's anatomy: The anatomical basis of clinical practice.41stedn. London: Elsevier, p.507.

2. Dhingra, V., Verma, J., Misra, V., Srivastav, S. and Hasan, F., 2017. Evaluation of cyclin D1 expression in head and neck squamous cell carcinoma. Journal of Clinical and Diagnostic Research: JCDR, 11(2), pp.1-4.
3. Gupta, N., Gupta, R., Acharya, A.K., Patthi, B., Goud, V., Reddy, S., Garg, A. and Singla, A., 2016. Changing Trends in oral cancer-a global scenario. Nepal journal of epidemiology, 6(4), pp.613-619.
4. Hossain, M.A., Ahmed, M.M., Rahman, A.S., Haider, M.N. and Alam, A.S., 2015. Epidemiological study of oral squamous cell carcinoma: A hospital based study in Dhaka city. Journal of Teachers Association, 28(2), pp.22-25
5. Talukder, L and Goswami, A., 2019. An immunohistochemical study of E-cadherin expression in primary Oral squamous cell carcinoma and metastatic lymph node in tertiary care centre. Pathology update: Tropical journal of pathology & Microbiology, 5(11), pp.884-891.
6. Bankfalvi, A., Kraßort, M., Végh, A., Felszeghy, E. and Piffkó, J., 2002. Deranged expression of the E-cadherin/β-catenin complex and the epidermal growth factor receptor in the clinical evolution and progression of oral squamous cell carcinomas. Journal of oral pathology & medicine, 31(8), pp.450-457.
7. Kudo, Y., Kitajima, S., Ogawa, I., Hiraoka, M., Sargolzaei, S., Keikhaee, M.R., Sato, S., Miyauchi, M. and Takata, T., 2004. Invasion and metastasis of oral cancer cells require methylation of E-cadherin and/or degradation of membranous β-catenin. Clinical Cancer Research, 10(16), pp.5455-5463.
8. Luo, S.L., Xie, Y.G., Li, Z., Ma, J.H. and Xu, X., 2014. E-cadherin expression and prognosis of oral cancer: a meta-analysis. Tumor Biology, 35(6), pp.5533-5537.
9. López-Verdín, S., de la Luz Martínez-Fierro, M., Garza-Veloz, I., Zamora-Perez, A., Grajeda-Cruz, J., González-González, R., Molina-Frechero, N., Arocena, M. and Bologna-Molina, R., 2019. E-cadherin gene expression in oral cancer: Clinical and prospective data. Medicina oral, patologia oral y cirugiabucal, 24(4), p.e444.
10. Ren, X., Wang, J., Lin, X. and Wang, X., 2016. E-cadherin expression and prognosis of head and neck squamous cell carcinoma: evidence from 19 published investigations. OncoTargets and therapy, 9, p.2447
11. Tasoulas, J., Giaginis, C., Patsouris, E., Manolis, E. and Theocharis, S., 2015. Histone deacetylase inhibitors in oral squamous cell carcinoma treatment. Expert Opinion on Investigational Drugs, 24(1), pp.69-78.
12. Chen, C. J., Hsu, L. S., Lin, S. H., Chen, M. K., Wang, H. K., Hsu, J. D., et al. (2012). Loss of nuclear expression of Krüppel-like factor 4 is associated with poor prognosis in patients with oral cancer. Human Pathology, Vol 43, pp. 1119–1125.
13. Liu, L.K., Jiang, X.Y., Zhou, X.X., Wang, D.M., Song, X.L. and Jiang, H.B., 2010. Upregulation of vimentin and aberrant expression of E-cadherin/β-catenin complex in oral squamous cell carcinomas: correlation with the clinicopathological features and patient outcome. Modern Pathology, 23(2), pp.213-224.
14. Monteiro, LS., Delgado,ML.,Ricardo,S., Amaral, B do., Salazar, F., Pacheco, JJ., Lopes, CA., Bousbaa, H., Warnakulasuryia, S., (2016)Oral Diseases, Vol.22, pp.303–312.
15. Zhou, J., Tao, D., Xu, Q., Gao, Z. and Tang, D., 2015. Expression of E-cadherin and vimentin in oral squamous cell carcinoma. International journal of clinical and experimental pathology, 8(3), p.3150
16. Zhong, L.P., Li, J., Zhang, C.P., Zhu, H.G., Sun, J. and Zhang, Z.Y., 2007. Expression of E-cadherin in cervical lymph nodes from primary oral squamous cell carcinoma patients. Archives of Oral Biology, 52(8), pp.740-747.
17. Khan, H.R., Patil, B.U. and Gangane, N.M., 2022. E-cadherin as a Prognostic Biomarker in Oral Squamous Cell Carcinoma: A Pilot Study at Tertiary Care Hospital. Medical Journal of Dr. DY Patil Vidyapeeth, 15(4), p.501.
18. Kadeh, H., Saravani, S. and Moghaddam, E.M., 2021. Immunohistochemical Expression of Epithelial Mesenchymal Transition Proteins in Squamous Cell Carcinoma of the Oral Cavity. Iranian journal of pathology, 16(4), p.354.
19. Warnakula suri S., 2009.'Global Epidemiology of Oral and Oropharyngeal Cancer'. Oral Oncology, 45 (4), pp. 309-316.
20. Sharma, J., Bhargava, M., Aggarwal, S., Aggarwal, A., Varshney, A. and Chopra, D., 2022. Immunohistochemical evaluation of E-cadherin in oral epithelial dysplasia and squamous cell carcinoma. Indian Journal of Pathology and Microbiology, 65(4), p.755.

21. Kumar, M., Nanavati1, R., Modi, TG., ChintanDobariya, C., (2016) Oral cancer: Etiology and risk factors: A review.Journal of Cancer Research and Therapeutics, Vol. 12, pp. 458-463.
22. Kushwaha, S.S., Joshi, S., Arora, K.S., Kushwaha, N.S., Sharma, S. and Saini, D.S., 2019. Correlation of E-cadherin immunohistochemical expression with histopathological grading of oral squamous cell carcinoma. Contemporary Clinical Dentistry, 10(2), p.232.
23. Mehendiratta, M., Solomon, M.C., Boaz, K., Guddattu, V. and Mohindra, A., 2014. Clinico-pathological correlation of E-cadherin expression at the invasive tumor front of Indian oral squamous cell carcinomas: An immunohistochemical study. Journal of oral and maxillofacial pathology: JOMFP, 18(2), p.217.