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EXPRESSION OF E- CADHERIN, β – CATENIN AND VIMENTIN IN PREMALIGNANT AND MALIGNANT LESIONS OF ORAL CAVITY AND OROPHARYNX – A 2-YEAR STUDY IN A TERTIARY CARE HOSPITAL

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ABSTRACT

BACKGROUND: Detection of high-risk cases for invasiveness at an early stage is necessary for the better prognosis of premalignant and malignant lesions of the oral cavity and oropharynx by determining the expression of E-Cadherin, β-catenin and Vimentin biomarkers. Hence, this study was conducted to study the histomorphology and the expression of E-cadherin, β-catenin and Vimentin along with the aim to assess the Immunohistochemistry (IHC) in regard to metastatic potential among the histopathological specimens of these lesions.
OBJECTIVES: To assess the histomorphology and the expression of E-cadherin, β-catenin and Vimentin along with the aim to assess the Immunohistochemistry (IHC) with regard to metastatic potential among the histopathological specimens of Premalignant and Malignant lesions of Oral Cavity and Oropharynx.
MATERIALS AND METHODS: Biopsy specimen from 87 cases of premalignant and malignant lesion from oral cavity and oropharynx were collected and staining of the slides with hematoxylin and eosin (H & E) was performed. Immunohistochemistry (IHC) was employed to assess the protein expression of E-cadherin, β-catenin and Vimentin in the Premalignant and Malignant lesions.
RESULTS: A total of 87 cases were subjected to Immunohistochemical study. 54.1% of the cases were within the age group of 41-60 years. Males constituted 64.4% and females 35.6% of the cases studied. Strong positivity expression of - E-Cadherin was found in 81.4%, β-Catenin in 81.7% and Vimentin in 85.7% of the total cases studied. Leucoplakia 79.6%was the commonest premalignant lesion and moderately differentiated squamous cell carcinoma 67.4% was the commonest malignant lesion encountered . E–Cadherin, β-Catenin and Vimentin showed 100% positivity in well differentiated and poorly differentiated squamous cell cancer. E–Cadherin, β-Catenin and Vimentin showed 100% positivity in Erythroleucoplakia.
CONCLUSION: Our study demonstrated that combined evaluation of the expression of E- Cadherin and Vimentin may provide a better indication of prognosis in patients with surgically resected oral squamous cell cancer. In future, multicentre studies would help a long way in establishing this association.

KEYWORDS

E-Cadherin, β – catenin, Vimentin, Oropharynx.

INTRODUCTION

Oral Cavity includes labial mucosa, buccal mucosa, floor of mouth, alveolar ridge and gingiva, anterior two-thirds of the tongue (anterior to the circumvallate papillae), hard palate, and retromolar trigone. Oropharynx includes soft palate, base (posterior one-third) of tongue, palatine tonsils, palatoglossal folds, vallecula and posterior pharyngeal wall[1]

The Head & Neck Squamous Cell Carcinoma (HNSCC) has been estimated to be responsible for 529,500 new cases and 292,300 death annually in 2012 accounting for about 3.8% of all cancer cases and 3.6% of cancer deaths, and predicted to rise by 62% to 856,000 cases by 2035.[2]

Oral Squamous Cell Carcinoma (OSCC) is one of the leading cancers in most Asian countries with global distribution and it ranks sixth most common neoplasm in the world [3] and ranks among the top three cancers in India.[4]

In North-East (NE) India, HNSCC creates really a public health problem with highest in the country 54.48% incidence.[5]

Potentially malignant lesions are precancerous lesions like Leukoplakia, Erythroplakia, Erythroleukoplakia and pre-cancerous conditions- Oral submucosal fibrosis, Actinic keratosis, Lichen planus, Siderophagic dysphagia, Discoid lupus erythematous, Palatal lesions in reverse cigar smoking, Syphilis, Dyskeratosis congenital, Epidermolysis bullosa and immunosuppression.[6]

There have been numerous attempts to develop tumor markers to determine the susceptibility of transformation of normal cells to malignant cells. Malignant transformation in many carcinomas is

associated with the loss of epithelial differentiation and gain of a mesenchymal phenotype; a process known as Epithelial to Mesenchymal Transition (EMT).[7]The epithelial markers identified during EMT associated with tumor progression are E-cadherin, β-Catenin and Vimentin.[8]E-cadherin is an important calcium-dependent transmembrane glycoprotein (cell adhesion molecule and signal transduction factor) located in the epithelial tissue. It can direct the formation of protein complexes attached to the actin cytoskeleton in combination with β-catenin formation which can prevent and reduce tumor cell adhesion. Hence, E-cadherin is an important symbol of occurrence of the loss of EMT.[9]

On the other hand, Vimentin is a cytoskeletal protein, not expressed in normal epithelial cells. The abnormal expression of Vimentin was also observed in a variety of epithelial tumors, and had close relationship with differentiation, invasion and metastasis of cancer cell. Herein, the role of angiogenesis in early stages of carcinogenesis is still unclear.[10]

This study was conducted to study the histomorphology and the expression of E-cadherin, β-catenin and Vimentin along with the aim to assess the Immunohistochemistry (IHC) in regard to metastatic potential among the histopathological specimens of Premalignant and Malignant lesions of Oral Cavity and Oropharynx.

MATERIALS AND METHODS

The study was a hospital based cross-sectional study of two years

duration from September 2018 to August 2020 conducted in the department of Pathology in collaboration with department of Otorhinolaryngology, Surgery and Dental College, Regional Institute of Medical Science (RIMS) Imphal, Manipur.A total of 87 biopsy specimen cases of suspected Premalignant and Malignant Lesions of Oral Cavity and Oropharynx registered in Department of Otorhinolaryngology, Department of Surgery and Dental College, Regional Institute of Medical Science, Imphal.were included in the study. Ethical approval from the institution and informed consent from the patients were taken before the initiation of the study. The tissue thus obtained was formalin fixed, paraffin embedded and stained with haematoxylin and eosin (H&E). After confirming the histopathological diagnosis, all cases were subjected to immunohistochemistry for E-Cadherin, b- Catenin and Vimentin expression. Both H&E and IHC slides were studied using light microscopy and the percentage and intensity of immunostaining was assessed. All the test were interpreted with negative and positive controls. Normal oral mucosal tissue was used as a positive control for Vimentin and breast tissue was used as a positive control for b-catenin and E-cadherin. Immunoreactivity was assessed semiquantatively on the basis of staining intensity and the following criteria was used for scoring.

Qualitative		Quantitative	
Score	**Pattern**	**Score**	**Pattern**
0	No Staining	0	No Staining or negative
+1	Weak Staining	+1	Staining < 10% of tumor cells
+2	Moderate Staining	2+	Staining 11-50 % of tumor cells
+3	Strong Staining	3+	Staining 51-80% of tumor cells
		4+	Staining > 80% of tumor cells

The extent of staining was estimated in percentage by counting at least 50 nuclei, calculating the ratio of reactive nuclei and/or cytoplasm to total number of nuclei and multiplying it by 100 and rounded off to the nearest 10%. The immunoreactive score combined the intensity and proportion score (Immunoreactive score=intensity score × proportion score). The intensity score was defined as 0 = negative; 1 = weak; 2= moderate; or 3= strong, and the proportion score was defined as 0 = negative; 1= <10%; 2 = 11–50%; 3 = 51–80%; or 4=>80%.

Descriptive and inferential analysis was carried out and results on categorical variables like gender were presented as frequency and percentages. Continuous variables like age were presented as mean and standard deviation. Chi-square test was used to determine the association between proportion and independent samples t-test was used for comparison of means. A p-value of < 0.05 was considered as statistically significant.

RESULTS

All 87 patients referred with premalignant or malignant lesions of the oral cavity and oropharynx were subjected to Immunohistochemical study.

Table 1. Age distribution of the study participants (N=87)

	Frequency (n)	Percentage
Age in years (Mean ± SD)	**57.5 ± 11.2**	
Age category (years)		
≤ 40	7	8.0
41-60	47	54.1
>60	33	37.9
Total	87	100

Above table 1 shows that the mean age of the patients was 57.5 years. Majority of the patients 54.1% were found to be in the age group of 41-60 years.

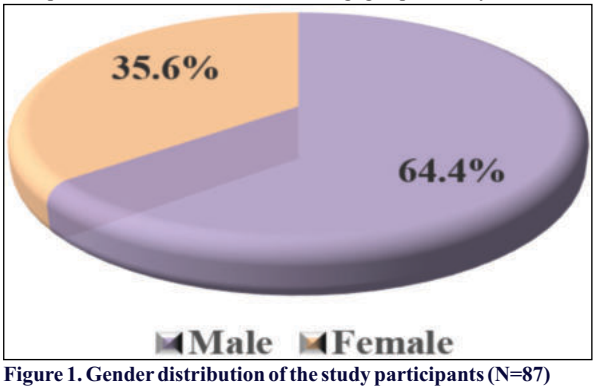

Figure 1. Gender distribution of the study participants (N=87)

Above figure 1 shows that 64.4% of the patients were male and 35.6% were female.

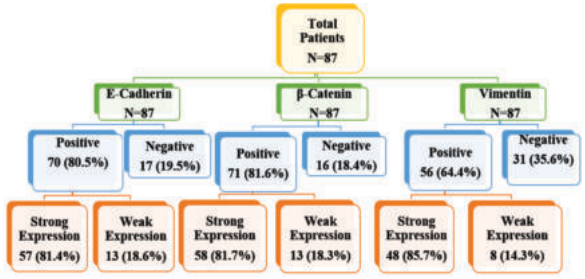

Figure 2. Distribution of patients by immunohistochemistry (N=87)

Above figure 2 shows the distribution of patients based on immunohistochemistry results. It can be noted that E-Cadherin was positive in 80.5% of the patients and β-Catenin and Vimentin were positive in 81.6% and 64.4% patients respectively. The positivity expression was strong in 81.4%, 81.7% and 85.7% among those who were positive for E-Cadherin, β-Catenin and Vimentin respectively.

Table 2. Distribution of participants by type of premalignant lesion (N=44)

Type	Frequency (n)	Percentage
Leucoplakia	35	79.6
Erythroplakia	7	15.9
Erythroleucoplakia	2	4.5
Total	44	100.0

Table 03. Site of the tumour and the positivity status of the biomarkers of SCC among the study participants (N=87)

Site	Marker positive		
	E-Cadherin n (%)	β-Catenin n (%)	Vimentin n (%)
Border of tongue	24 (88.9)	24 (88.9)	18 (66.7)
Buccal mucosa	17 (77.3)	17 (77.3)	10 (45.5)
Palate (Hard and Soft)	6 (60.0)	6 (60.0)	5 (50.0)
Gingiva + Alveolar	6 (66.7)	6 (66.7)	5 (55.6)
Floor of mouth	6 (100.0)	6 (100.0)	6 (100.0)
Tonsil	2 (50.0)	3 (75.0)	3 (75.0)
Vocal cord	3 (100.0)	3 (100.0)	3 (100.0)
Base of tongue	3 (100.0)	3 (100.0)	3 (100.0)
Glottis	3 (100.0)	3 (100.0)	3 (100.0)
Total	70	71	56

Above table 03 shows that the positivity status which was higher for E-Cadherin and β-Catenin for all the sites. There was a slight reduction in positivity status for Vimentin for tumours at buccal mucosa, palate and gingiva.

Table 04. Association of type of tumour with E-Cadherin among the study participants (N=87)

Type of tumour	E-Cadherin		Total	p value
	Positive n (%)	Negative n (%)		
Malignant	41 (95.3)	2 (4.7)	43	0.001
Pre-malignant	29 (65.9)	15 (34.1)	44	

Above table 04 shows that E-Cadherin positivity was significantly higher among those with malignant lesions when compared to those with pre-malignant lesions (95.3% vs 65.9%; p=0.001).

Table 05. Association of type of tumour with β-Catenin among the study participants (N=87)

Type of tumour	β-Catenin		Total	p value
	Positive n (%)	Negative n (%)		
Malignant	42 (97.7)	1 (2.3)	43	0.001
Pre-malignant	29 (65.9)	15 (34.1)	44	

Above table 05 shows that β-Catenin positivity was significantly higher among those with malignant lesions when compared to those with pre-malignant lesions (97.7% vs 65.9%; p<0.001).

Table 06. Association of type of tumour with Vimentin among the study participants (N=87)

Type of tumour	Vimentin		Total	p value
	Positive n (%)	Negative n (%)		
Malignant	39 (90.7)	4 (9.3)	43	0.001
Pre-malignant	17 (38.6)	27 (61.4)	44	

Above table 06 shows that Vimentin positivity was significantly higher among those with malignant lesions when compared to those with pre-malignant lesions (90.7% vs 38.6%; p<0.001).

DISCUSSION

Epithelial markers like E- Catherin, b- Catenin and Vimentin may play a role in tumour progression. More than half (54.1%) of the patients were in the age group of 41-60 years in our study and it is similar to the studies conducted by Akhtar K et al11 in which the most common age group was found to be 40-60 years. Nearly 64.4% of the patients with the oral squamous cell carcinoma in our study group were males which is consistent with other studies and the temporal association is well established by various other studies added to the fact that it's quiet common for the males to use tobacco more when compared to the females.[11]

Our study showed that cancer at the border of the tongue and buccal mucosa to be the common sites for the oral cancer. The findings are in accordance with other studies conducted across the globe.[12] Our study also showed higher positivity rate of E-Cadherin, β-Catenin and Vimentin among the patients with malignant lesion when compared to the patients with the pre-malignant lesion and it was found to be statistically significant. One of the molecules related to cell adhesion is β-Catenin, with a dichotomous effect: it is able not only to actively participate in the process of intercellular adhesion but also related to intracellular signals transduction. Hence, the association β-Catenin is biologically plausible. Similar kind of association was observed by various other studies. The positive association for the decreased expression of E-Cadherin was reported by various bother studies.[12] Similarly increased expression of Vimentin by the cancerous lesions have been reported by other studies similar to our study findings.[12] The association for β-Catenin was similar to the study conducted by Lopes FF et al[13]

One of the strengths of our study is that it was done on a representative sample of a population and hence, it could be generalised to the similar settings,and combined evaluation of E-cadherin and vimentin along with β-Catenin adds more strength to our results. One of the limitations is that PCR could have been used for the specific diagnosis of the markers. Information regarding lifestyle habits such as smoking or alcohol and betel consumption, which may be the confounding factors, was not available.

CONCLUSION

Our study also showed higher positivity rate of E-Cadherin, β-Catenin and Vimentin among the patients with malignant lesion when compared to the patients with the pre-malignant lesion and it was found to be statistically significant and also demonstrated that combined evaluation of the expression of these proteins as a measure of EMT may provide a better indication of prognosis in patients with surgically resected OSCC. In future, multicentre studies would help a long way in establishing this association.

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