Original Research Paper

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Oral Pathology

AN IMMUNOHISTOCHEMICAL STUDY OF E-CADHERIN, VIMENTIN AND KI-67 EXPRESSION IN RESPECT TO CLINICOPATHOLOGICAL PROFILE OF ORAL SQUAMOUS CELL CARCINOMA IN A TERTIARY CARE CENTRE

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ABSTRACT **INTRODUCTION:-** According to WHO, Oral cancer is one of the three most prevalent cancers in Asia, among which India accounts for approximately one third. Immunological markers E-cadherin, Vimentin and Ki-67 plays a major role in determining invasiveness, metastasis and prognosis of Oral Squamous Cell Carcinoma. **AIM:-** To study the expression of immunological markers E-cadherin, Vimentin and Ki-67 in Oral Squamous Cell Carcinoma and evaluate their expression concerning clinicopathological profile. **METHODS:-**It is a hospital-based cross-sectional study of 60 cases of Oral Squamous Cell Carcinoma done for two years. Expression of immunological markers E-cadherin, Vimentin and Ki-67 were evaluated and correlated with its pathology. **RESULTS:-** Majority of the patients with Oral Carcinoma belonged to the 51 – 60 years age group with male predominance(2.3:1). 45% had on Tongue, 22% on the buccal mucosa, 12% on Hard palate, 8% on Lips, 5% on Soft palate and Uvula,3% on Anterior pillar and Retromolar trigone and 2% on Floor of mouth. E-cadherin was positive in 72% with 38% strong positivity. Vimentin was positive in 33% with 20% strong positivity. Ki-67 expression was high in 67% of the cases. Lymph node metastasis showing 13% E-cadherin positivity , 22% Vimentin positive, 30% Ki-67 positive. **CONCLUSION:-** OSCC cell proliferation research must be further extended as it can be helpful to forecast survival rates for a deeper understanding of protein expression and the interaction between biomarker and therapeutic aspects of these patients.

KEYWORDS : E-cadherin, Vimentin, Ki-67, OSCC

INTRODUCTION

According to the World health organization (2018), one of the three most prevalent cancers in Asia and the Pacific is oral cancer (Carcinoma of the lips and mouth)[1] with India accounting for approximately one-third of the total burden and oral cancer is the sixth most prevalent cancer worldwide[2]. Tobacco, alcohol and usage of the areca nut are all known to cause oral cancer[3]. An increasing number of young persons in North America and Europe are developing oral malignancies due to Human papillomavirus infection[4]. India is home to roughly 77,000 new cases and 52,000 fatalities a year, which is about one-fourth of the worldwide incidences[5]. Oral squamous cell carcinoma (OSCC) is a major cause of oral cancer, accounting for 84-97 percent[5].

The epithelial to mesenchymal transition (EMT), which is common in Oral squamous cell carcinoma, involves the loss of epithelial morphology and the development of mesenchymal features[6]. Many epithelial markers including E-cadherin, desmoplakin, cytokeratin and claudins, are downregulated in cells prior to EMT[6]. E-cadherin is an intracellular domain that interacts with its cytoplasmic binding partner catenin to connect it to the actin cytoskeleton[7]. Vimentin is a marker for cells that are in transition from epithelial to mesenchymal features[8]. Ki-67 is a proliferation marker for cells. Ki-67 overexpression is also utilized to determine if a patient is at high risk of malignant transformation and for grading Oral squamous cell carcinoma[9]. In this research, immunohistochemistry was used to assess the expression of epithelial E-cadherin, Vimentin and Ki-67 in Oral squamous cell carcinoma, as well as its grading concerning invasion, metastasis and cellular proliferation.

AIMS AND OBJECTIVES:-

1. To evaluate Immunohistochemical expression of E-cadherin, Vimentin and Ki-67 in Oral Squamous Cell Carcinoma.
2. To assess the role of these markers to determine invasion, metastasis and cellular proliferation.

METHODOLOGY:-

This study is a hospital based cross-sectional study done on 60 cases of Oral Squamous Cell Carcinoma done for a period of 2 years from (November 2018- November 2020). Sampling method:-All the diagnosed cases presented to the hospital were included. Inclusion criteria:- positive biopsy specimens of Oral Squamous Cell Carcinoma with adequate tissue for analysis. Exclusion criteria:- Squamous Cell Carcinoma with inadequate tumour tissue and Patients not willing to participate. Method:-The oral biopsy specimens were preserved in a 10% formaldehyde solution. Immunohistochemistry was used to evaluate the expression of E- cadherin, and histological diagnosis was made on haematoxylin and eosin-stained sections. Ethics: ethical clearance was obtained from the institution, and informed consent was obtained from participants.Analysis:-The data was collected, coded and entered in Microsoft excel, and the excel sheet was exported to IBM SPSS software (21 edition trial version) for analysis. The results are presented in the form of frequencies and percentages. The Chi-square test was applied wherever necessary. A P-value of less than 0.05 was considered significant.

RESULTS:-

Table no.1 shows, a majority (33%) of the study population belonged to the age group of 51– 60yrs and the Male: Female ratio was 2.3:1. Out of 60 cases, 45% of the cases had Carcinoma Tongue. 22% of cases had Carcinoma on Buccal mucosa. 12% of the cases had Carcinoma on the Hard palate. 8% of the cases had Carcinoma on lip.

Table No.1 Distribution Of Age Gender, Site Of Cancer Tumor Grade And Lymph Node Metastasis Among Study Population

VARIABLES	FREQUENCY	PERCENTAGE	
AGE	31 - 40	9	15.00%
	41 - 50	11	18.33%
	51 - 60	20	33.33%
	61 - 70	12	20.00%
	>70	8	13.33%
GENDER	Male	42	70%
	Female	18	30%
SITE	Lip	5	8.33%
	Buccal mucosa	13	21.66%
	Tongue	27	45.00%
	Floor of mouth	1	1.66%
	Retromolar trigone	2	3.33%
	Hard palate	7	11.66%
	Soft palate, Uvula	3	5.00%
	Anterior pillar	2	3.33%
Tumour Grade	Well Differentiated	42	70%
	Moderately Differentiated	14	23%
	Poorly Differentiated	4	7%
Lymph node Metastasis	Present	22	36.66%
	Absent	38	63.33%

Well Differentiated Tumour was found in 70% of the cases. Moderately Differentiated Tumour was found in 23% of the cases. Poorly Differentiated Tumour was found in 7% of the cases. Out of 60 Cases, lymph node metastasis was present in 22 (37%) of the cases and

in 38 (63%) of the cases, lymph node metastasis was not found.

Table No.2 Distribution Of-cadherin And Vimentin Expression In Relation With Tumor Grade

TUMOUR GRADE	E-CADHERIN		VIMENTIN	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
WELL-DIFFERENTIATED	35(56.66%)	7(13.33%)	8(13.33%)	34(56.66%)
MODERATELY DIFFERENTIATED	7(13.33%)	7(13.33%)	9(15%)	5(8.33%)
POORLY DIFFERENTIATED	1(1.66%)	3(5%)	3(5%)	1(1.66%)
P-VALUE	0.005	0.01		

Table no.2 shows, E-cadherin was positive in 57% of the Well Differentiated and 13% of the Moderately Differentiated Tumour cases. Vimentin was positive in 13% of the Well Differentiated and 15% of the Moderately Differentiated Tumour cases. The difference between the groups was found to be statistically significant (p-value <0.05).

Table No.3:distribution Of Ki-67 Expression In Relation With Tumor Grade

TUMOUR GRADE	Ki-67		
	LOW	BORDERLINE	HIGH
WELL-DIFFERENTIATED	4(6.6%)	17(28.3%)	21(35%)
MODERATELY DIFFERENTIATED	1(1.6%)	2(3.3%)	11(18.3%)
POORLY DIFFERENTIATED	2(3.3%)	1(1.66%)	1(1.6%)
TOTAL	7	20	33
P-VALUE	0.04		

Table no.3 shows Ki-67 expression was high in 35% of Well Differentiated Tumours, borderline in 28.3% of the Well Differentiated Tumours, and low in 6.6% of the Well Differentiated Tumours. Ki-67 expression was high in 1.6% of the Poorly Differentiated Tumours, borderline in 1.66% of the Poorly Differentiated Tumours and low in 3.3% of the Poorly Differentiated Tumours.

FIGURES

FIGURE 1-4.A case of Well Differentiated Carcinoma showing strong positivity of E-cadherin, Vimentin negative and Ki-67 positive only in basal layer of epithelium.

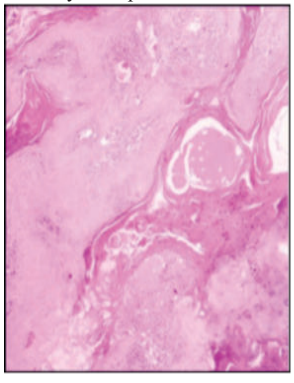

Fig 1:H&E(10X)

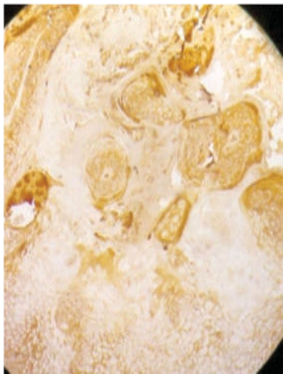

Fig 2: E-cadherin(10X)- Strong positive

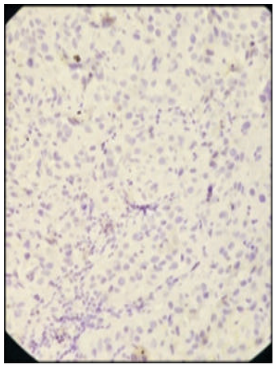

Fig 3: Vimentin(40X) -Negative

Fig 4: Ki-67(10X)- Focal positive only in basal layer

Figure 5-8 : A case of Moderately Differentiated Squamous Cell Carcinoma, showing weak positivity of E-cadherin, weak positivity of Vimentin and high Ki-67 positivity.

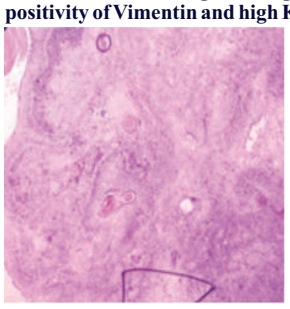

Fig 5: H&E(10X)

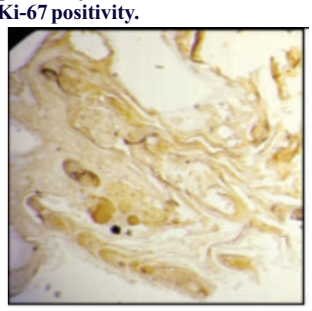

Fig 6: E-cadherin(10x) weak positive

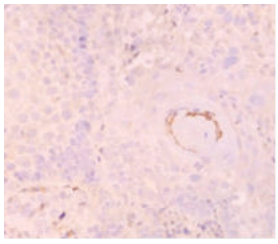

Fig 7: Vimentin(40x) weak positive

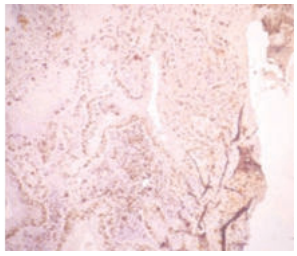

Fig 8: Ki-67(10X) high proliferative index

Figure 9-12:-A case of Poorly Differentiated Carcinoma showing negative E-cadherin, positive Vimentin, high Ki-67 positivity

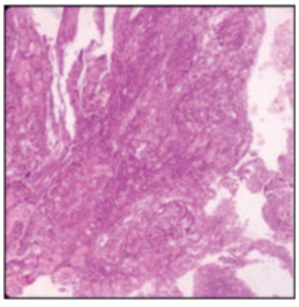

Fig9 :H&E(10X)

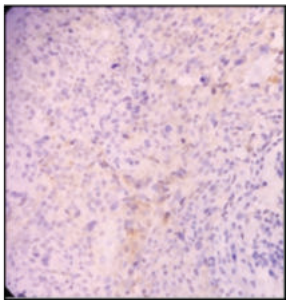

Fig10 :E-cadherin(40X)-negative

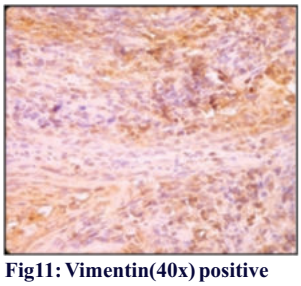

Fig11: Vimentin(40x) positive

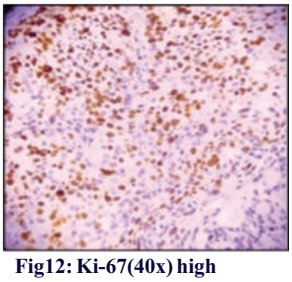

Fig12: Ki-67(40x) high proliferative index

DISCUSSION:-

In the present study, male predominance was observed. Our study results were identical to CL. Margaritescu et al.10 Patel M et al.11, Marwah et al.study12 also reported similar results with males and females contributing 75 per cent and 25 per cent, respectively. In this present study, the majority of the patients with Oral Carcinoma belonged to the age group of 51 - 60 yrs with 33%.In a study done by Jing ping et al.13 compared with age group and E-cadherin also Vimentin positivity and observed that the majority of patients over 50 years of age were E-cadherin and Vimentin positive. The p values measured were not important for either age group and marker positivity correlation.

In the present study, a majority of cases had Carcinoma Tongue followed by Carcinoma of Buccal mucosa. This finding was also seen in similar studies done by Subha Bhat et al.14 and CA Fischer et al.15.

In this study, the majority of cases were Well-Differentiated, followed

by Moderately-Differentiated followed by Poorly-Differentiated histological form. Similar findings were seen in CL. Margaritescu et al.10, but another study by Marwah et al.12 showed more Moderately Differentiated histological form.

In the present study, more than half the cases had lymph node metastasis; among them, only 22% of the cases were Vimentin positive and 13% E-cadherin positive. Similar findings were seen Jing ping et al.13. Thus, the lymph node metastasis statistical correlation shows increased Vimentin expression and diminished E-cadherin expression. In this study, a correlation was made with the advancing age group and the expression of E-cadherin and Vimentin. The positivity of E-cadherin was found to be more in Well and Moderately Differentiated, the expression of E-cadherin was decreased or absent in Poorly Differentiated Tumours, and the expression of E-cadherin was negative or lost in those tumours with invasive nature and positive for lymph node metastasis. Vimentin was statistically significant in Poorly Differentiated Tumours with increased positivity and lymph node metastasis.

Ki-67 is a predictor for cell proliferation in multiple tumours. The activity of Ki-67 tumour proliferation was established in connection with tumour aggression, which is determined by the tumour grade and stage. Many studies have represented these relations and established Ki-67 as a forecasting factor. The results of this study support that Ki-67 is a widely articulated independent predictor for OSCC. In consideration of the large variety of tumours, more studies need to improve spatial associations between Ki-67 and OSCC.

CONCLUSION:-

This present study demonstrated the relationship of E-Cadherin, Vimentin and Ki-67 in forecasting the possibility of invasion and metastasis and prognosis of the patients. 66.6% of the cases showed a high proliferation index which is regarded as a poor prognostic indicator and increased chances of recurrence. Ki-67 is an established prognostic, predictive and survival protein marker. Its expression is higher in Moderately Differentiated and Poorly Differentiated Squamous Cell Carcinoma. So this relationship of E-cadherin, Vimentin and Ki-67 helps in forecasting the possibility of invasion, metastases and prognosis of an individual. In conclusion, OSCC cell proliferation research must be further extended as it can be helpful to forecast survival rates for a deeper understanding of protein expression and the interaction between biomarker and therapeutic aspects of these patients.

Abbreviations:- OSCC- Oral squamous cell carcinoma, E-Cadherin - epithelial cadherin.

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