Original Research

Expression of caveolin 1 in oral squamous cell carcinoma

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Abstract

Context: Caveolin-1 is a surface protein that is a major structural component of caveolae, which are vesicles of the plasma membrane integral to a variety of signal transduction molecules and transport functions. Caveolin-1 is a biomarker undergoing research & studies have shown an increased expression of Cav-1 in the stepwise carcinogenesis from the normal oral mucosa, hyperplastic mucosa, dysplastic mucosa, precancerous lesions to Oral Squamous Cell Carcinoma. In the present study Correlation between Caveolin-1 expression and grade of tumor was established statistically.

Aims: To study immunohistochemical expression of Caveolin-1 in Oral Squamous Cell Carcinoma.

Settings and Design: Cross sectional study carried out in a tertiary care hospital.

Materials and Methods: A total of 90 cases of histopathologically diagnosed oral squamous cell carcinoma was evaluated. Grading of the cases into well, moderate and poorly differentiated carcinomas was done as per WHO guidelines . Margin and lymph node status were evaluated. Anti- Caveolin-1 antibody (E249)-Caveolae marker ab32577 was used in the dilution of 1:100. Results were expressed taking reference of the methodology used by Hung et al 2003.

Statistical Analysis Used: Statistical Package for the Social Sciences (SPSS 25.0).

Results: Correlation of tumor grade and lymph node metastasis was statistically significant $p=0.0006$. There was a significant statistical correlation between tumor grade and immunohistochemical expression of Caveolin-1, p- value=0.00. Correlation between Lymph node metastasis and Caveolin-1 was statistically significant, p-value=0.008.

Conclusions: Caveolin-1 expression correlates with aggressive tumor behavior and poor prognostic outcome.

Keywords: Caveolin-1, oral cancers, carcinogenesis

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INTRODUCTION

Oral cancers are the sixth most common cancer in the world.[1] Oral cancer is a major public health problem in the Indian subcontinent, where it ranks among the top three types of cancer in the country.[2] The low-income groups in India are affected most due to wide exposure to risk factors such as tobacco chewing and insufficient exposure to new diagnostic aids, resulting in a delay in the reporting of oral cancer.[3,4]

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It is estimated that more than 90% of all oral cancers are oral squamous cell carcinoma (OSCC).[5] Depending on the site and extent of the involvement of cancer and the type of treatment modality, these patients experience pain, trismus, xerostomia, dysphagia and taste disturbances compromising them socially and nutritionally.[6]

Despite the advances in diagnostic as well as therapeutic approaches, the percentage of morbidity and mortality of OSCC has not improved significantly during the last 30 years. The percentage of five-year survival for patients with OSCC varies from 40% to 50%.[7]

Because of the increased prevalence of oral cancer and poor survival for patients with oral cancers, finding a reliable biomarker for the prediction of the progression and prognosis of OSCC is of paramount importance.

Caveolin-1 is a surface protein that is a major structural component of caveolae, which are vesicles of the plasma membrane integral to a variety of signal transduction molecules and transport functions. Research has shown CAV-1 to act as a scaffolding protein by directly influencing a multitude of signalling cascades including Src-family tyrosine kinases and the RAS family.[8-10] This ability to modulate signalling has produced significant evidence to suggest that overexpression or downregulation of caveolae may have important roles in the process of cell transformation and tumour formation.[11,12]

Caveolin 1 is a biomarker undergoing research, and studies have shown an increased expression of Cav-1 in the stepwise carcinogenesis from the normal oral mucosa, hyperplastic mucosa, dysplastic mucosa, precancerous lesions to oral squamous cell carcinoma (OSCC).[13]

The purpose of prognostic studies in early-stage cancer is to identify a subset of patients who are at risk of adverse outcomes and will therefore need more appropriate treatment, such as multimodality therapy, in contrast with another subset of patients who have increased chances of a favourable outcome.

MATERIALS AND METHODS

We evaluated a total of 90 cases of histopathologically diagnosed OSCC of buccal mucosa, alveolus, anterior two-thirds of the tongue, gingivobuccal sulcus, hard and soft palate, the floor of the mouth and retromolar trigone in the study. Among the 90 patients, 74 were males and 16 females with the mean age of presentation being 55.6 years.

Squamous cell carcinoma from sites other than the oral cavity diagnosed pre-cancerous lesions of the oral cavity and verrucous carcinoma of oral cavity were excluded from the study.

10% neutral buffered formalin was used as a fixative of choice. Specimens were received after careful examination of patients' demographic and clinical details along with details of surgical procedures performed. The specimens were examined grossly, serially sectioned and fixed in 10% neutral buffered formalin for 12–24 hours.

Grading of the cases into well, moderate and poorly differentiated carcinomas was done as per WHO guidelines based on the degree of differentiation, cellular pleomorphism and mitotic activity as recommended in CAP guidelines.[14] Margin and lymph node status were evaluated.

For studying caveolin expression, standard operating procedure for IHC was followed:

Anti-Caveolin-1 antibody (E249) – Caveolae marker ab32577 – was used in the dilution of 1:100.

Caveolin 1 immunoreactivity was normally localised to fibroblasts and endothelial cells of blood vessels which served as an internal control.

Results were expressed taking reference of the methodology used by Hung *et al.* 2003.[13] In our study, we graded the percentage of immunopositive cells as follows: [Table 1]

RESULT

In the present study, 43/90 (47.78%) were moderately differentiated squamous cell carcinoma, followed by 42/90 (46.67%) of well-differentiated squamous cell carcinoma and 5/90 (5.56%) of poorly differentiated squamous cell carcinoma.

In our study, the majority, 29/43 (67.4%), of moderately differentiated squamous cell carcinoma and 3/5 (60%) of poorly differentiated squamous cell carcinoma showed lymph node metastasis. Among 42 cases of well-differentiated squamous cell carcinoma, only 26.19% showed lymph node metastasis.

The correlation of tumour grade and lymph node metastasis was statistically significant in the present study $P = 0.0006$ [Table 2].

In the present study, the majority, 4/5 (80%), of the poorly differentiated carcinomas expressed high

immunohistochemical expression for caveolin 1. In moderately differentiated squamous cell carcinoma, 23/43 (53.48%) showed low immunohistochemical expression for caveolin 1 followed by 17/43 (39.53%) showing intermediate immunohistochemical expression for caveolin 1 [Figure 1 and Table 3].

There was a significant statistical correlation between tumour grade and immunoexpression of caveolin-1, *P* value = 0.00.

Out of the seven cases with high immunohistochemical expression of caveolin-1, 5/7 (71.42%) showed lymph node metastasis, whereas 16/22 (72.72%) cases with intermediate immunoreactivity showed lymph node metastasis and only 22/61 (36.06%) cases with low immunoreactivity showed lymph node metastasis. The correlation between Lymph node metastasis and caveolin was statistically significant, *P* value = 0.03 [Table 4].

DISCUSSION

Oral cancer accounts for 2%-4% of all cancer cases worldwide with a prevalence of around 45% in India, Nepal, Pakistan and Sri Lanka.[1,15] Tobacco is the main etiologic factor for oral cancers. Tobacco is used in various forms in Southeast Asia including the Indian subcontinent in the form of betel[15] quid, tobacco with lime, bidi etc., Other minor factors like HPV[16] infection, dietary deficiencies and poor oral hygiene[17,18] also contribute as etiologic agents in OSCC. Oral cancers often go unnoticed in the early stages and patients often present in an advanced stage of the disease. In spite of the vast amount of research and advances in oral cancers, there is significant morbidity and mortality associated with it.

Table 1: Grading of immunohistochemical expression of caveolin-1

Low	<10% of tumor cells show immunohistochemical expression of caveolin-1
Intermediate	10-50% of the tumor cells show immunohistochemical expression of caveolin-1
High	>50% of the tumor cells show immunohistochemical expression of caveolin-1

Table 2: Correlation of tumour grade and lymph node metastasis

Tumour grade	Lymph node metastasis *n*=90			Pearson Chi-square value	*P*
	Involved	Free	Total		
Well	11	31	42 (46.7%)	14.81	0.0006
Moderate	29	14	43 (47.8%)		
Poor	3	2	05 (5.5%)		
Total	43	47	90 (100%)		

*Significant when *P*<0.05

Table 3: Correlation of immunohistochemical expression of caveolin 1 with tumour grade

Tumour grade	Caveolin 1				Pearson Chi-square value	*P*
	High	Intermediate	Low	Total		
Well	0	4	38	42 (46.67%)	52.58	0.000
Moderately	3	17	23	43 (47.78%)		
Poor	4	1	0	5 (5.56%)		
Total	7	22	61	90 (100%)		

*Significant when *P*<0.05

Table 4: Correlation of immunohistochemical expression of caveolin 1 with lymph node metastasis

Lymph node metastasis	Caveolin 1				Pearson chi-square value	*P*
	High	Intermediate	Mild	Total		
Involved	5	16	22	43 (47.8%)	10.41	0.0340
Free	2	6	39	47 (52.2%)		
Total	7	22	61	90 (100%)		

*Significant when *P*<0.05

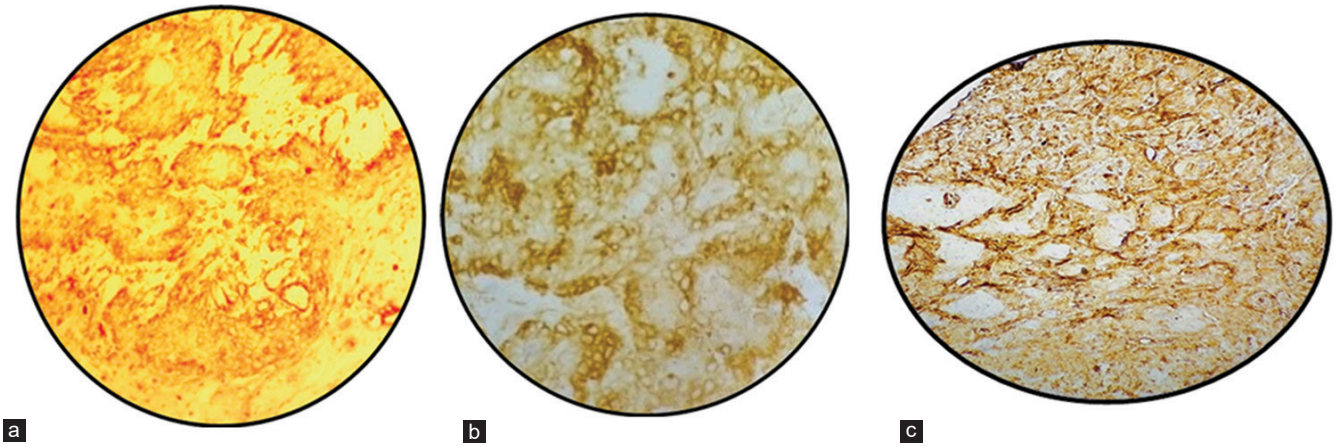

Figure 1: (a) Photomicrograph showing low immunohistochemical expression of Caveolin-1 in Well differentiated Oral Squamous Cell Carcinoma(100x), (b) Photomicrograph showing Intermediate immunohistochemical expression of Caveolin-1 in Moderately differentiated Oral Squamous Cell Carcinoma(400x), (c) Photomicrograph showing high immunohistochemical expression of Caveolin-1 in Poorly differentiated Oral Squamous Cell Carcinoma (100x)

Histologic grading has been used as a prognostic factor and for clinical behaviour evaluation of OSCC for the past several decades.[19]

In our study, there were 42 (46.67%) cases of well-differentiated squamous cell carcinoma, 43 (47.78%) cases of moderately differentiated carcinoma and 5 (5.56%) cases of poorly differentiated carcinoma. Our findings are consistent with the findings of Deval Parekh *et al.* 2020[20] who reported more number (67.36%) of cases with moderately differentiated tumour.

The presence of cervical lymph node metastasis in patients with head and neck carcinomas leads to poor prognosis. In patients with nodal metastasis, the five-year survival rate has been reported to be 20%–36% after surgical treatment as compared to 63%–86% in patients with no lymph node involvement.[21]

Immunohistochemistry is an emerging prognostic tool in oral cancers.

Caveolin-1 was first identified as a tyrosine-phosphorylated protein in Rous sarcoma transformed cells[22] that was enriched in both caveolae[23] and vesicles targeted to the apical surface of polarized epithelial cells.[24] Caveolin-1, a multifunctional protein, is the main constituent molecule of caveolae and represents a scaffolding molecule for several signalling molecules.

When described in relation to OSCC, studies were found confirming that an increased CAV 1 expression plays an important role in the process of carcinogenesis and development of tumour.[25]

In our study immunohistochemical expression of caveolin 1 with tumour grade was statistically significant (*P* value = 0.0).

Our findings were in concordance with the findings of Nakatani *et al.* 2005,[26] who observed a significant correlation between tumour grade and caveolin expression ($P = 0.027$) and Xue *et al.* 2010[27] who also reported statistical significance between expression of caveolin and tumour grade ($P < 0.05$).

We also found a significant statistical correlation between caveolin expression and lymph node metastasis (*P* value = 0.01) which was concordant with the study done by Hung *et al.* 2003[11,13] (*P* value = 0.004).

CONCLUSION

Caveolin 1 expression correlates well with the tumour grade and lymph node metastasis. Thus, caveolin 1 expression correlates with aggressive tumour behaviour and poor prognostic outcome.

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Conflicts of interest
There are no conflicts of interest.

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