ORIGINAL ARTICLE

Grading of Cox-2 expression in oral squamous cell carcinoma – An immunohistochemical study

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Introduction: Oral cancer is a major health issue in South-east Asia. The immunohistochemical expression of cyclooxygenase-2 (COX-2) in oral squamous cell carcinoma is well seen. Many evidences showed that role COX-2, an enzyme that catalyzes the synthesis of prostaglandins, has an important pathology in carcinogenesis. In many studies, it has been seen that selective COX-2 inhibitors inhibit the growth formation of tumors including carcinomas of oral cavity. The immunohistochemistry (IHC) was done to evaluate the COX-2 expression in different grades of oral squamous cell carcinoma and comparing it with normal mucosa. **Objectives:** The aim of the study was to determine the role of COX-2 expression as a screening marker in oral squamous cell carcinomas (OSCC). **Materials and Methods:** All histopathologically confirmed cases of oral squamous cell carcinoma (45 cases of OSCC and 10 cases of Normal mucosa) and positive control colon cancer were studied for the expression of IHC. Out of 45 cases, 22 cases, 15 cases, and eight cases were of well, moderate, and poorly differentiated carcinomas were studied. COX-2 expression was done on the basis of positive and negative tumors cells and intensity of staining of tumor cells. **Results:** The study showed that COX-2 expression was seen in all the cases of squamous cell carcinoma expressed COX-2. Intensity varied among different grades of OSCC. Statistically significant high expression of COX-2 was seen in increasing grades of OSCC. No expression of COX-2 was seen in normal mucosa. Our study concluded that COX-2 enzymes play an important role in oral carcinogenesis and thereby in the future administration of chemoradiation therapy combined with COX-2 can help in improving therapy response.

KEY WORDS: Cyclooxygenase-2, Immunohistochemistry, Oral squamous cell carcinoma

INTRODUCTION

Oral cancer is most common malignant neoplasm in the world wide. It accounts for approximately 2% of all cancer and 1% of all cancer deaths, it is a global health problem with rising incidence and mortality. In India, oral cancer represents a leading problem constituting up to 42% in males and 18% in females.[1] Despite in recent advance in surgery, radiotherapy, and chemotherapy, the annual cancer death for squamous cell carcinoma of oral cavity is rising rapidly.

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Oral squamous cell carcinomas (OSCC) are cancers originating from the squamous epithelium in the oral cavity locations include the lip, tongue, buccal mucosa, labial mucosa, floor of the mouth, gingiva, hard palate, and soft palate. OSCC belongs mainly to subgroup of tumors termed head-and-neck squamous cell carcinoma, comprising carcinoma originating in the oral cavity, oropharynx, larynx, hypopharynx, nasal cavity, nasopharynx,

paranasal sinuses, and salivary glands. The male citizens have habitually a higher incidence in OSCC, ordinarily 2:1 compared to women. This is, however, due to increased alcohol consumption and tobacco use among men,squamous cell carcinoma of oral cavity has a multifactorial etiology, the cause of oral cancer in the Western world is the use of tobacco and alcohol.[2] In addition to this, there are many other factors which predispose to cause oral cancer, these are poor oral hygiene among low socioeconomic people, viral infections that are left untreated, occupational exposure, and malnutrition as diet deficient in fruits and vegetable and genetic factors have been proposed for the development of oral cancer.

Cyclooxygenase (COXS) catalyze the synthesis of prostaglandins from arachidonic acid. There are two isoforms of cox. One is expressed constitutively (COX-1) and the other is inducible (COX-2). [3,4]

The COX-2 gene is an early response gene that is induced by growth factors, oncogenes, carcinogens, cancer causing phorbol esters, several cytokines, hypoxia, and ultraviolet radiation and it is expressed in many neoplastic processes, it stimulates cell division, angiogenesis, and inhibits apoptosis.[4,6]

Many evidences from different experimental systems shared that COX-2 has an important role in carcinogenesis. COX-2 is up regulated in transformed cell[3-7] in malignant tumors.[6,8] Cox-2 an enzyme plays a role in synthesis of prostaglandin which is important in pathway of carcinogenesis. Prostaglandin especially of E series promotes cell proliferation, invasion angiogenesis, and metastasis.

COX-2 has been shown to be upregulated in various types of cancers including those arising from colon, stomach, breast, lung, esophagus, pancreas, bladder, prostate, and OSCC. Use of non-steroidal anti-inflammatory drugs decreases the risk of several malignancies including SCC.

Microscopically histological tumor grading into well differentiated keratinizing squamous cell carcinoma, moderately differentiated keratinizing squamous cell carcinoma, poorly differentiated keratinizing squamous cell carcinoma as per the 2017 latest WHO classification of head-and-neck.[9] COX-2 Immunohistochemistry (IHC) expression in the tumors was noted according to the grading system followed by Mohammad *et al*.[10]

In this study, we intend to investigate the immunohistochemical expression of COX-2 in squamous cell carcinoma of oral cavity compared with normal mucosa and there by elucidate their involvement in oral carcinogenesis.

Inclusion Criteria

All the clinically diagnosed biopsy specimen of oral squamous cell carcinoma received during the study period of (1 year).

Exclusion Criteria

Specimen reported as inadequate, patient who are already receiving treatment and patient on chemotherapy.

METHODOLOGY

A total of 45 cases of OSCC and 10 cases of normal mucosa and positive colon cancer were studied for the expression of COX-2. Of 45 cases (22 cases of well differentiated,15 cases of moderately differentiated, and eight cases of poorly differentiated carcinoma) were studied.

After approval of our institutional review board, formalin fixed paraffin embedded (FFPE) tissue blocks of histologically diagnosed cases of OSCC were taken.

Immunohistochemical Interpretation

After FFPE tissue blocks were taken up for IHC according to the manufacturer's recommended protocol. COX-2 IHC staining was done by COX-2 (SP-21) primary antibody.

Control

FFPE tissue blocks of histologically proven cases were retrieved from the archives of Department, including colon carcinoma.

Prepared slides were viewed under optical microscope 40× magnification, for positive or negative immunostaining.

The staining was interpreted to the cytoplasm and perinuclear area.

The positivity will be expressed in terms of percentage of tumor cells positive for COX-2 expression and intensity of staining.

Negative Staining[10]

No cell stained.

Positive Staining

- GRADE 1<5%
- GRADE 25–30%
- GRADE 3 >30%

Negative and <5% COX-2 positivity will be taken as negative expression and >5% COX-2 positivity as positive expression group. The >30% COX-2 positivity will be taken as overexpressed group and <30% COX-2 positivity as under expressed.[10]

Intensity of Staining

The positive result will be assessed further for intensity of staining as mild, moderate, and intense in different grades of OSCC.

RESULTS AND OBSERVATIONS

Carcinoma colon was taken as positive control showing COX-2 positivity [Figure 1]. All normal mucosa do not show expression of COX-2.

The staining intensity and number of positive tumors cells were evaluated. Increase in staining intensity was high in poorly

differentiated carcinoma to well differentiated and moderately differentiated OSCC. Poorly differentiated show maximum staining [Figures 2-4].

Table 1 shows the distribution of patients according to grade of COX2. Grade 2 COX2 was among more than one third of patients (44.4%) followed by Grade 3 (28.9%) and Grade 1 (26.7%).

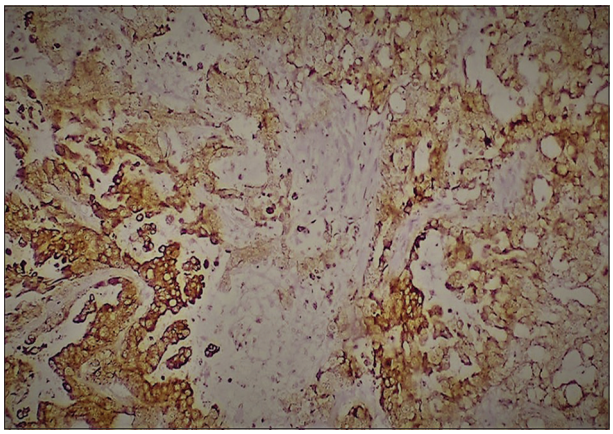

Figure 1: Colon carcinoma (positive control showing COX-2 positivity)

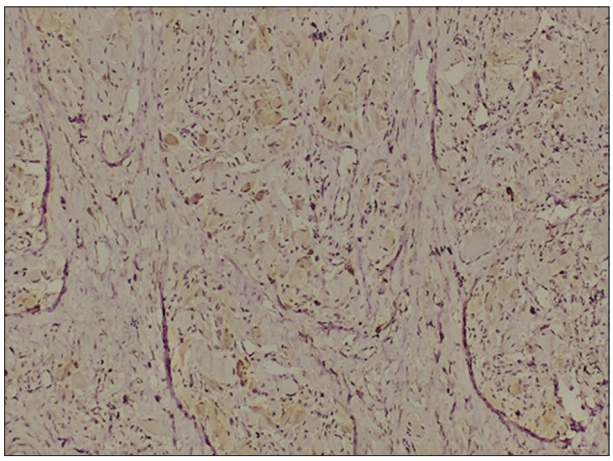

Figure 2: Section showing Grade 1 COX-2 positivity in well-differentiated squamous cell carcinoma

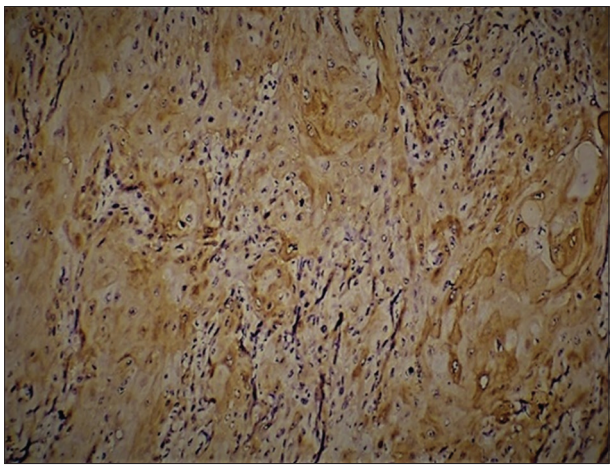

Figure 3: Section showing Grade 2 COX-2 positivity in moderately differentiated squamous cell carcinoma

Table 2 shows well differentiated carcinoma express Grade 1 COX-2 expression (54.5%), moderately differentiated carcinoma express Grade 2 COX 2 expression (66.7%), and poorly differentiated carcinoma expressed Grade 3 COX-2 (100.0%), there was increase in staining intensity from well to poorly differentiated.

DISCUSSION

In our study, distribution of patients according to COX-2 status and COX 2 grading was done, it was found that all the histologically diagnosed cases of oral squamous cell carcinoma showed the expression of COX 2 where as normal mucosa which was used for the comparison do not show any expression, COX 2 staining was divided into three grades according to the staining intensity of tumor cells: Grade 1 <5%, Grade 2 5–30%, and Grade 3 >30%, this study shows accordance with Thomas *et al*.[11] in which 30 cases of oral squamous cell carcinoma and 10 cases of normal mucosa were taken and it was found that there was significant increase in staining intensity in cases of OSCC. No significant expression of COX-2 observed in normal mucosa, this study was also similar to Chan *et al*.[12] and Atula *et al*.[13] which show expression of COX 2 in oral squamous cell carcinoma and its correlation with advancement of these, Pandey *et al*.[14] also had a similar study that showed increase expression of COX 2 in dysplastic epithelia and its overexpression in squamous cell carcinoma play an important part in early stage of carcinogenesis as well as tumor progression, this study shows discordance with the study of Shibata *et al*.[15] in which COX 2 expression was also seen in normal mucosa and there was increase expression of COX-2 in oral dysplasia compared with oral squamous cell carcinoma.

Table 1: Distribution of patients according to grade of COX2

Grade of COX2	No. (*n*=45)	%
Grade 1	12	26.7
Grade 2	20	44.4
Grade 3	13	28.9

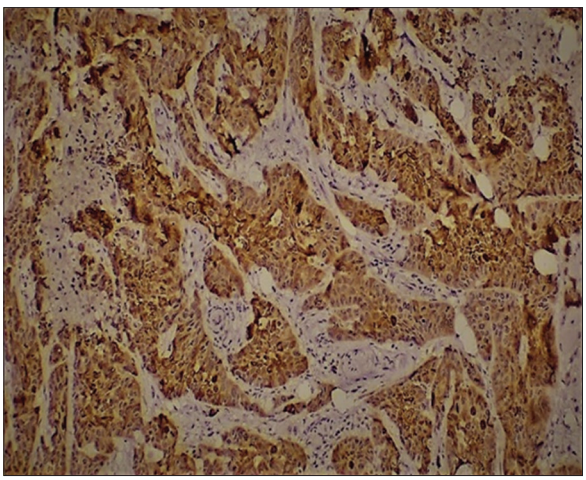

Figure 4: Section showing Grade 3 COX-2 positivity in poorly differentiated squamous cell carcinoma

Table 2: Association of SCC with grades of COX-2

SCC	No. of patients	Grade 1		Grade 2		Grade 3		*P*-value[1]
		No.	%	No.	%	No.	%	
Well differentiated	22	12	54.5	10	45.5	0	0.0	NA
Moderately differentiated	15	0	0.0	10	66.7	5	33.3	
Poorly differentiated	8	0	0.0	0	0.0	8	100.0	

[1]Chi-square test, NA-Not applicable as>1 0s in a column

In our study, COX-2 expression was correlated with the histological grades of oral squamous cell carcinoma in this study intensity of staining increases from well differentiated to poorly differentiated carcinomas, poorly differentiated carcinoma shows increase staining intensity, this study suggest that COX-2 may be useful indicator of the malignant phenotype of squamous cell. In our study, results were that most commonly well differentiated carcinoma express Grade 1 COX-2 expression (54.5%) moderately differentiated carcinoma express Grade 2 COX 2 expression (66.7%) and poorly differentiated carcinoma expressed Grade 3 COX-2 (100.0%), there was increase in staining intensity from well to poorly differentiated carcinoma, this study shows accordance with Nagatsuka *et al.,*[16] statistical significance was seen between COX-2 expression and histological grade, this findings was like to Sappayatosok *et al*.[17] which suggest that there is constant increase in the staining intensity as the tumor grade increased from well to poorly differentiated carcinoma. These findings show dis-similarities with the study of Shamma *et al*.,[18] in their study, the expression of COX-2 increased from normal to low-grade dysplasia and was highest in severe dysplasia but it is gradually decreases from primary cancer to advance SCC, they show the inverse correlation between COX 2 and histological differentiation of OSCC.

CONCLUSION

Our study revealed a difference in immunohistochemical staining of COX-2 in different grades of OSCC and normal mucosa, thereby showing the role of COX-2 in pathway of carcinogenesis. It shows that COX-2 inhibitors could be used in preventing premalignant transformation to malignant lesions.

Our study suggests that tumor cells of squamous cells carcinomas of oral cavities express COX-2. Intensity might vary according to the sub groups, it was found that expression was high in more advanced grade of carcinoma, where as normal mucosa did not show any expression of COX-2. We recommend future research studies on administration of chemoradiation therapy combined with COX-2 to evaluate improvement in therapy response.

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