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Original Research Article

Correlation of Cyclo-Oxygenase-2 (COX-2) Expression with Tumor Differentiation and Lymphovascular Invasion in Cervical Cancer

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Abstract

Background: Cyclo-Oxygenase-2 (COX-2) derived prostaglandins are known to participate in carcinogenesis, tumor cell invasion and metastasis. COX-2 is expressed in all stages of cancer and it's over expression is associated with poor prognosis. The purpose of the present study was to correlate COX-2 expression with tumor differentiation and lymphovascular invasion in cervical cancer. *Materials and Methods:* Fifty one surgically resected hysterectomy specimens and cervical biopsy specimens sent for histopathology department in a tertiary care hospital were included in the study. Formalin-fixed paraffin-embedded tissue sections were stained by Hematoxylin and Eosin. Immunohisto-chemistry for COX-2 was also performed on these blocks. *Statistical analysis used:* Chi square test and Fisher Exact test were applied to test the significant associations, where $p < 0.05$ was considered as statistically significant. *Results:* Of the 51 specimens, 6 (11.8%) were found to be Cervical intra epithelial neoplasia (CIN), 40 (78.4%) were Squamous cell carcinoma (SCC), 4 (7.9%) were Adenocarcinoma and 1 (1.9%) was of poorly differentiated carcinoma variant. Though majority of the specimens were of invasive carcinomas (45), microscopic evidence of Lymphovascular invasion was seen only in 27 (60%) cases. All adenocarcinoma and poorly differentiated carcinoma cases and some (22) SCC cases showed lymphovascular invasion. Intensity of COX-2 expression was lower in in-situ carcinomas compared to invasive carcinomas. It was found that higher intensity of COX-2 expression was significantly associated with higher tumour grade and lymphovascular invasion. ($p < 0.05$) *Conclusion:* In the present study, intensity of COX-2 expression was found to be varying with the tumour grade and lymphovascular invasion in carcinoma cervix. This demonstrates the role of COX-2 as a prognostic marker in cervical carcinomas.

Keywords: Cyclo-Oxygenase-2; Carcinoma of cervix; Lymphovascular invasion; Tumour differentiation.

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Introduction

Cervical cancer is the fourth most commonly diagnosed cancer in women. In 2018, about 5,70,000 new cases were detected contributing to 6.6% of all female cancers as per WHO. Human papilloma virus (HPV) is an important cause of most common histologic type of cervical cancer. It is also seen that women who are smokers, immune-compromised or having chronic inflammation have an increased risk of developing cervical cancer in the setting of HPV.[1,2,3] Hence, these observations have led to the investigation of immune system and immunomodulators in the development of cervical cancer. Cyclo-oxygenase (COX) enzymes are responsible for conversion of Arachadonic acid to prostaglandins which are key components of inflammation.[4] While COX-1 isoform is expressed constitutively, COX-2 isoform is induced in the presence of inflammatory stimulus.[5] The relation between COX-2 and chronic inflammation is studied extensively and reported that there is close relation between Cyclooxygenases and cervical cancer. The carcinogenic effect is because of the ability of COX-2 to resist apoptosis, stimulate tumour cell proliferation, tumour metastasis, inhibit immune surveillance.[6] Until now, only a few studies have been undertaken to evaluate the role of COX-2 in carcinomas of cervix. In this study we shall evaluate the role of COX-2 in carcinomas of cervix by studying the expression pattern in different types of carcinomas.

Aims:

The purpose of the present study was to correlate COX-2 expression with tumor differentiation and lymphovascular invasion in cervical cancer

Materials and Methods

This prospective study was conducted from June 2015 to May 2017, in the department of Pathology of urban tertiary care centre, on the surgically resected hysterectomy specimens and cervical biopsy specimens in 10% formalin received from department of Obstetrics and Gynaecology and surgical oncology for routine histopathological examination.

After obtaining ethical clearance, by purposive sampling, a total of 51 specimens were included for the study. This sample size was calculated based on the findings of a study which showed that, 25% of cases which did not show lymphovascular invasion were positive for COX-2, whereas it was 77% of cases that showed lymphovascular invasion. Assuming an alpha error of 5% and relative precision of 15%, sample size was calculated to be 51 cases of carcinoma cervix.

All hysterectomy specimens and cervical biopsy specimens from female patients of all age groups with in-situ/invasive cervical carcinoma were included for the study. Cervical biopsies which were inadequate for complete evaluation, with any other primary malignancies of cervix, metastatic tumors in the cervix, and malignancies with extensive tumor necrosis and inflammation were excluded.

Cases of colon carcinoma were taken as positive controls, as internal negative control, tumor unchanged epithelial cells of the cervix were used. After conventional processing, paraffin sections of 5 μm thickness were stained by haematoxylin and eosin (H & E) for histopathological study. In addition, 4 μm sections were be cut from a paraffin block of tumor tissue and taken on 4 glass slides coated with adhesive (polyL-lysine) for immunohistochemistry (IHC) to detect COX-2 expression. Immunohistochemical detection of COX-2 expression was done on 4 μm thick sections, cut from a paraffin block of tumor tissue and taken on a glass slide coated with adhesive (poly-Lysine). Positive and negative controls were run with each batch of slides. Specimens were evaluated microscopically, and semi-quantitative analysis was done, where the extent of cytoplasmic and membrane COX-2 expression was graded and scored. The intensity of staining was marked as strong (+2, +3, +4), weak (+1) and negative (0).

Statistical data analysis was performed with the aid of the software package IBM SPSS 18.0. Data analysis was done and presented as tables and graphs. Chi-square test and Fisher Exact test were applied to test the significant associations, where $p < 0.05$ was considered as statistically significant.

Results

In the present study, out of the 51 specimens, 6 cases (11.9%) were reported as cervical intra epithelial neoplasia (CIN), 40 (78.4%) cases as Squamous cell carcinoma (SCC), 4 (7.8%) cases as Adeno-carcinoma and only 1 (1.9%) was reported as poorly differentiated carcinoma (PDC) (Table 1).

Table 1: Distribution of the specimens based on the Histological type (n=51)

Histological Type	Frequency	(%)
Cervical intra epithelial lesion	6	11.9
Squamous cell carcinoma	40	78.4
Adenocarcinoma	4	7.8
Poorly differentiated carcinoma	1	1.9
Total	51	100

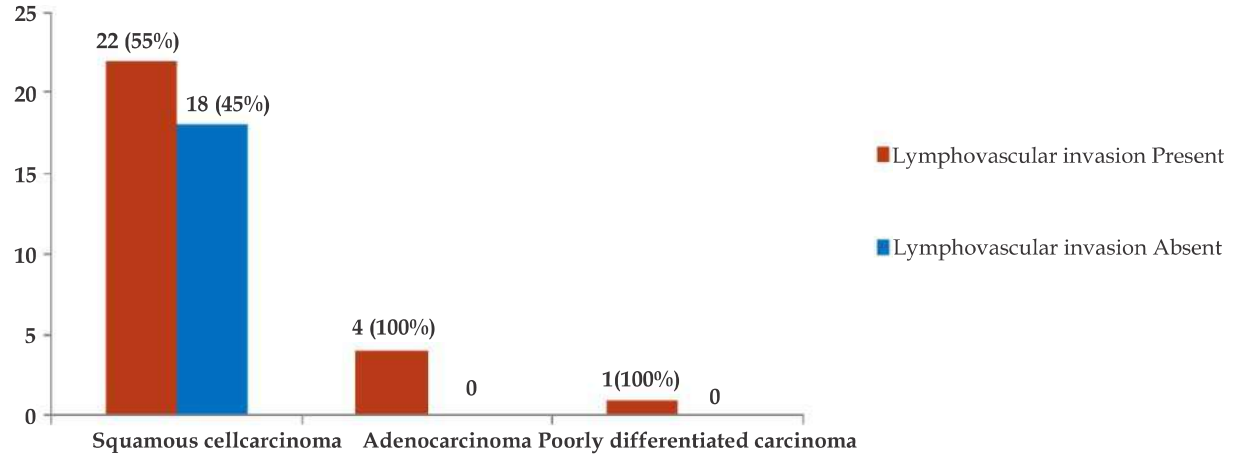

Fig. 1: Presence of Lymphovascular invasion among the invasive carcinomas (n=45)

Out of the remaining 45 cases of invasive carcinomas, lymphovascular invasion was seen in all the Adenocarcinoma and poorly differentiated carcinoma specimens (100%); whereas lymphovascular invasion was seen in only 22 (55%) cases of Squamous cell carcinoma (Fig. 1).

Table 2: Correlation of intensity of COX-2 expression with histological subtype (n=51)

Histological subtype	Intensity of COX-2 expression			Total
	+2	+3	+4	
Carcinoma in-situ	6 (100%)	0 (0%)	0 (0%)	6 (11.8%)
Squamous cell carcinoma	2 (5%)	15 (37.5%)	23 (57.5%)	40 (78.3%)
Adenocarcinoma	0 (0%)	0 (0%)	4 (100%)	4 (7.8%)
Poorly differentiated carcinoma	0 (0%)	0 (0%)	1 (100%)	1 (1.9%)
Total	**8 (100%)**	**15 (100%)**	**28 (100%)**	**51 (100%)**

Among the total specimens (51), the intensity of COX-2 expression was less in in-situ carcinoma (+2) as compared to Adenocarcinoma (+4) and Poorly differentiated carcinoma (+4) and this relation was found to be statistically significant ($p < 0.05$) (Table 2).

Table 3: Correlation of intensity of COX-2 expression with tumour grade (n=45)

Tumour grade	Intensity of COX-2 expression			Total	*p*-value
	+2	+3	+4		
Grade-II	2 (100%)	14 (93.3%)	10 (35.7%)	26 (57.8%)	
Grade-III	0 (0%)	1 (6.7%)	18 (64.3%)	19 (42.2%)	<0.001*
Total	**2 (100%)**	**15 (100%)**	**28 (100%)**	**45 (100%)**	

(*By Fisher Exact test, where p<0.05 is statistically significant)

Among the 45 invasive cervical cancer specimens 26 were of grade II and 19 were of grade III. Grade III tumors had higher intensity of COX-2 expression compared to grade II tumours and this result was statistically significant (Table 3).

Similarly, specimens with lymphovascular invasion had higher intensity of COX-2 expression, and this correlation was statistically significant ($p < 0.05$) (Table 4)

Table 4: Correlation of intensity of COX-2 expression with Lymphovascular invasion (n=45)

Lymphovascular invasion	Intensity of COX-2 expression			Total	*p*-value
	+2	+3	+4		
Present	1 (50.0%)	4 (26.7%)	22 (78.6%)	27 (60.0%)	
Absent	1 (50.0%)	11 (73.3%)	6 (21.4%)	18 (40.0%)	0.001*
Total	**2 (100%)**	**15 (100%)**	**28 (100%)**	**45 (100%)**	

(*By Fisher Exact test, where p<0.05 is statistically significant

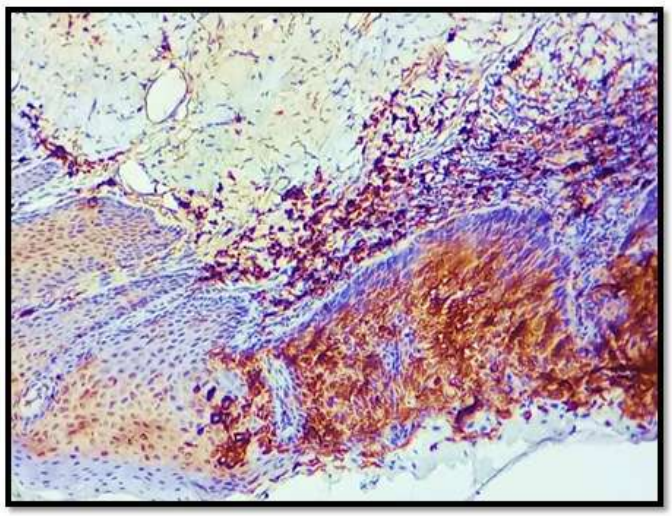

Fig. 2: COX-2 IHC expression in carcinoma in-situ of Cervix (40x)

The dysplastic squamous epithelium on the right side has taken up the COX-2 IHC stain compared to normal squamous epithelium on the left. COX-2 staining intensity of +2.

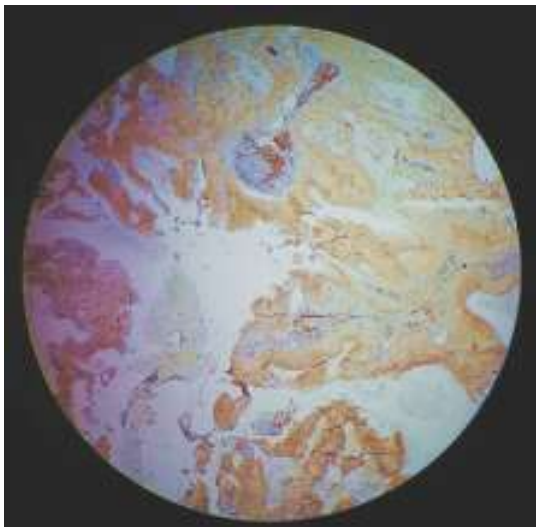

Fig. 3: COX-2 IHC of squamous cell carcinoma moderately differentiated-(10x) –staining intensity of +3.

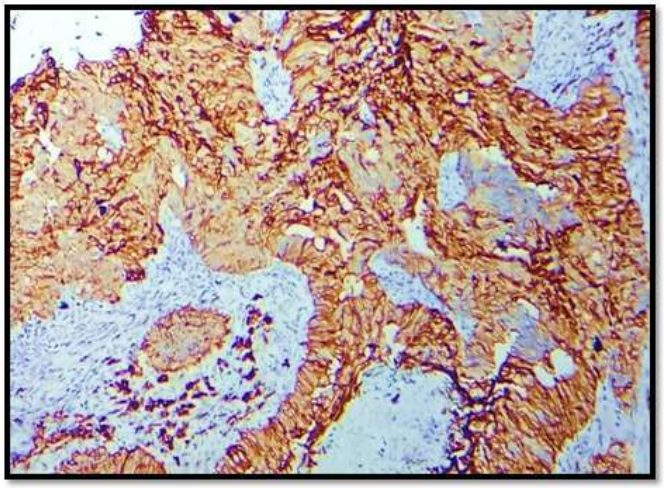

Fig. 3: COX-2 IHC staining of adenocarcinoma of cervix-40x magnification COX-2 staining intensity +4.

Discussion

It is well known that the concentration of prostaglandin in tumor tissues is much greater than the corresponding normal tissues. Cyclooxygenase, the key enzyme in the prostaglandin metabolism, has been studied extensively to find out its potential role in the development and progression of tumors. It has been noticed that COX-2 expression is involved in both early and late stages of carcinogenesis. Increased COX-2 expression has been demonstrated in high grade SCC of esophagus, adenomatous and metaplastic lesions of stomach, pre-neoplastic lesions of lung, pre-invasive neoplasias of breast, bladder, and pancreas.[5] Studies on the expression of COX-2 in cervical cancer are relatively few. It has been shown to be expressed in dysplastic epithelium and in invasive carcinomas.

Correlation of intensity of COX-2 expression with tumour grade

In the present study we noted that COX-2 staining intensity increased with the grade of the tumour. Highest intensity (+4) was seen Grade III (poorly differentiated) tumours compared to Grade II (moderately differentiated) and Grade I (well differentiated) tumours. These results were statistically significant. Similar results were shown by Balan[7] *et al*. and Fukazawa[8] *et al*. But according to Bandyopadhyay[5] *et al*., these results were not statistically significant.

Correlation of intensity of COX-2 expression with squamous cell carcinoma and adenocarcinoma

COX-2 expression has also been demonstrated in Adenocarcinomas of cervix. In the present study adenocarcinomas of cervix showed a stronger positivity (+4) in all 4 cases as compared to squamous cell carcinoma and in higher percentage of cases. But this difference was not statistically significant. The number of cases might have been very small to draw a conclusion from this. According to Manchana[9] *et al*. COX-2 expression in Cervical adenocarcinoma was higher than squamous cell carcinoma (86.7% vs 40.6%; $p<0.05$), and it was concluded that a strong correlation exists between Cervical Adenocarcinoma and Cox-2 expression. Similarly Fathima[10] *et al*. analysed 130 cases of cervical carcinomas for COX-2 expression, which was found to be more prominent in Adenocarcinomas. A study by Jung YW[11] *et al*. showed that the Adenocarcinoma patients with higher COX-2 expression had unfavourable treatment response for the disease.

Correlation of intensity of COX-2 expression with lymphovascular invasion:

In the present study, out of the 45 invasive carcinoma cases, 27 cases (60%) had lymphovascular invasion and these cases had higher intensity of COX-2 expression. The remaining 18 cases which did not have Lymphovascular invasion had comparatively lesser intensity of COX-2 expression. This correlation was statistically significant with a p-value of <0.05. Similarly Khunamornpong[12] *et al*. showed that COX-2 expression was associated with Lymph node metastasis ($p = 0.007$) in cervical SCC, but this was also linked to the presence of lympho vascular space invasion. They concluded that COX-2 expression may enhance lymph node metastasis after lymphovascular space invasion occurs.

Similarly according to study by Bandyopadhyay[5] *et al*, positive association was found between the intensity of COX-2 expression and the presence of lymphovascular emboli.

In a similar study conducted by Mandic[13] *et al*, the correlation of COX-2 expression and the presence of lymphovascular invasion showed a statistically significant difference (presence of lymphovascular invasion in 61.9% of the patients with positive COX-2; absence of lymphovascular invasion in 33.3% and $p < 0.05$). This observation again points to the probable role of COX-2 expression as a prognostic marker and it may be used in conjunction with other major criteria in modifying the postoperative adjuvant therapy in SCC.[14,15] Only a few studies have evaluated the association between the COX-2 expression and tumour response in patients who have undergone radiotherapy[16].

Conclusion

Carcinoma cervix being one of the common cancers affecting the women, immunohistochemical profile with various markers shall help in diagnosis and shall also help in planning the therapeutic regime for the patients. COX-2 can be used as one such marker, as it is highly expressed in various types of cervical neoplasms, and can be associated clinically with cervical carcinoma development and progression. Accordingly, this study showed that the intensity of COX-2 expression increased with the severity of cervical dysplasia, increased tumour grade and with the presence of lymphovascular invasion. Further, studies with a larger number of cases can establish the therapeutic role of COX-2 inhibitors in cervical carcinomas.

Conflict of Interest: Nil

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