Original Article

Expression of Epidermal Growth Factor Receptor in Squamous Cell Carcinoma of Uterine Cervix

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Abstract

Context: Cervical cancer is one of the most important cancer deaths among females. Epidermal growth factor receptor (EGFR) plays a role in cell differentiation, cell motility, neovascularization, invasion, metastasis, and resistance of cancer cells to chemotherapeutic agents and radiation. Tyrosine kinase inhibitors and anti-EGFR monoclonal antibodies have shown better response in various invasive tumors. **Aims:** The aim of the study is to evaluate EGFR expression in squamous cell carcinoma (SCC) of the cervix and to assess its relation to tumor characteristics. **Settings and Design:** This was a retrospective, case–control study. **Subjects and Methods:** Formalin-fixed paraffin-embedded tissues from 30 cases with SCC along with 20 age-matched cases with normal cervix as controls were obtained from the archives. EGFR expression was analyzed in both cases and controls. **Statistical Analysis Used:** The Chi-square test was used to compare and find the association between the variables. Statistical analysis was done using the IBM SPSS (IBM, Armonk, NY, USA) software and $P < 0.05$ was considered significant. **Results:** Strong EGFR expression was present in 93.4% of the cases, while 6.6% of cases showed moderate expression. Strong EGFR expression was associated with the tumor size of >4 cm size. There was no association of EGFR expression with tumor grade, tumor stage, and lymph node metastasis. **Conclusions:** The present study showed that a significantly higher number of cases of invasive SCC of uterine cervix show increased EGFR expression. The EGFR expression is associated with tumor size.

Keywords: *Carcinoma, cervix, epidermal growth factor receptor, squamous*

Introduction

Cervical cancer is one of the most common causes of deaths related to cancer among females in developing countries.[1] Today, majority (80%) of the females dying due to cervical cancer live in developing countries.[2] It is curable in early stages, but prognosis for advanced stages of the disease is very poor.[3]

It arises in the form of precursor lesions, i.e., cervical intraepithelial neoplasia (CIN) which is closely associated with infection by various strains of human papillomavirus, namely 16, 18, 31, 13, 33, and 51. Genetic modifications leading to the expression of oncoproteins play an important role in the pathogenesis of tumors. Analysis of these oncoproteins may lead to discovery of potential targets for anticancer therapies.[4]

Radiation had been the gold standard therapy for cervical cancers for many decades. Currently, concurrent cisplatin-based chemoradiotherapy has been considered as the standard therapeutic modality for locally advanced cervical cancers.[5,6] However, such treatment options remain suboptimal with residual tumor observed in as much as 40%–50% of cases.[7] Patients presenting with recurrent or metastatic tumors have limited treatment options[3] and in such cases the 5-year survival is <5%.[8]

Epidermal growth factor receptor (EGFR) has been known to play a role in cell differentiation, enhancement of cell motility, protein secretion, neovascularization, invasion, metastasis, and resistance of cancer cells to chemotherapeutic agents and radiation.[9,10]

EGFR is a 170-kDa transmembrane glycoprotein receptor encoded by the HER1 proto-oncogene located on chromosome 7p12. EGFR is synthesized from a 1210 residue polypeptide precursor after cleavage of the N-terminal sequence; an 1186 residue protein is inserted into the cell membrane.[11]

How to cite this article: Yadav SK, Verma A, Sarin N, Singh S. Expression of epidermal growth factor receptor in squamous cell carcinoma of uterine cervix. Clin Cancer Investig J 2019;8:227-31.

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Access this article online

Website: www.ccij-online.org

DOI: 10.4103/ccij.ccij_69_19

Quick Response Code:

EGFR is present in many normal tissues and expressed in a wide variety of solid tumors. EGFR is normally expressed in the cytoplasm and the membrane of the cells in the basal layer of various epithelial membranes.[12] In cervical cancer, the range has been reported to be anywhere from 6% to 90% of cases.[13]

The current standard of cancer therapy is receptor-mediated tumor-targeted radiotherapy and chemotherapy, which is based on the delivery of therapeutically relevant drugs directly to disseminated tumor cells, with hopefully minimal damage to normal tissues.

Tyrosine kinase inhibitors such as erlotinib and newer targeted therapy drugs against EGFR receptors such as cetuximab and panitumumab have been approved for use in various primary and metastatic colon cancers[14-16] and nonsmall cell lung cancers.[17]

The expression of EGFR in cervical cancer has rarely been studied. The present study was carried out with the aim to evaluate the expression of EGFR oncoprotein in squamous cell carcinoma (SCC) of the cervix and to find out its association with various tumor characteristics and to compare the expression with normal control subjects.

Subjects and Methods

Formalin-fixed paraffin-embedded blocks of tissue from 30 cases with SCC of uterine cervix were obtained from the departmental histopathology archives. Twenty age-matched cases of normal cervix, removed for lesions other than those related to cervix (mostly leiomyoma) were taken as controls. Clinical and demographic details of all patients were retrieved from the records. Staging of the SCC cases was done according to the International Federation of Gynecology and Obstetrics (FIGO) staging system for cervical tumors.[2]

Sections were stained by hematoxylin and eosin staining for histological examination. Cases were graded into well-differentiated, moderately differentiated, and poorly differentiated SCC.[18]

Immunohistochemical staining was done on 5-µm tissue sections taken on poly-L-lysine coated slides. EGFR immunostaining was done using polymer-Horse-raddish peroxidase detection kit, (Biogenex, USA). For positive control, tissue from SCC of lung positive for EGFR was used. Level of EGFR expression was evaluated using a semi-quantitative method with regard to the intensity of staining and the percentage of positive tumor cells.[19] Intensity of staining was scored on a scale of 0–3 as: 0 (no color), 1 (slightly brown), 2 (brown), and 3 (dark brown). The percentage of positively stained cell was scored on a scale of 0–3 as: 0 (no stained cells), 1 (1%–<50% stained cells), 2 (51%–80% stained cells), and 3 (more than 80% stained cells). Intensity and percentage scores were added and final score was calculated using these score and four categories were identified as: negative (0), weak positive (1–2), moderately positive (3–4), and strongly positive (5–6). The Chi-square test was used to compare and find the association between the variables. Statistical analysis was done using the IBM SPSS (IBM, Armonk, NY, USA) software and $P < 0.05$ was considered statistically significant.

Results

The mean age of the study group was 48.13 ± 7.81 years and that of control group was 44.00 ± 7.52 years.

Cases were categorized according to tumor characteristics and shown in Table 1 and Figure 1. The tumor size was found to be <4 cm among 53.3%. Cases with moderately differentiated carcinoma were 66.7% followed by poorly differentiated (20%) and well differentiated (13.3%). The lymph, node, metastasis was found in 46.5% of the cases. FIGO stage IB1 was observed in 53.3% of the cases.

Table 1: Distribution of cases according to tumor characteristics

Tumor characteristics	*n* (%)
Tumor size (cm)	
<4	16 (53.3)
>4	14 (46.6)
Tumor grade	
Well differentiated	4 (13.3)
Moderate	20 (66.7)
Poor	6 (20.0)
Lymph node metastasis	
Positive	10 (33.3)
Negative	20 (66.7)
TNM stage	
T1b1 N0 M0	16 (53.3)
T1b2 N0 Mx	4 (13.3)
T1b2 N1 Mx	10 (33.3)
FIGO stage	
IB1	16 (53.3)
IB2	4 (13.3)
IIIB	10 (33.3)

TNM: Tumor, node, metastasis, FIGO: International Federation of Gynecology and Obstetrics

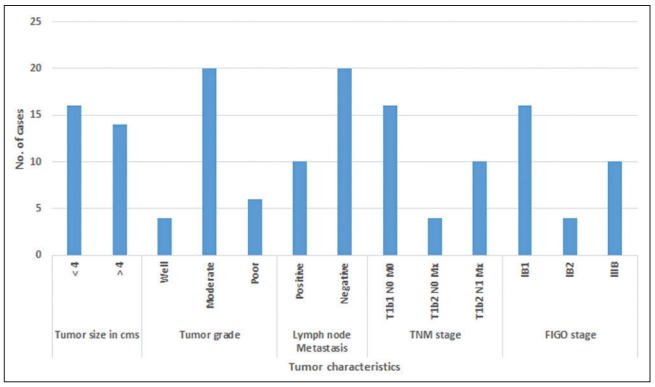

Figure 1: Distribution of cases according to tumor characteristics

EGFR immunoexpression was present in both cases and control groups. No case or control tissue was found as negative or weakly positive for EGFR expression. Moderate expression (2+) was seen in 6.6% cases, while strong expression was present in 93.4% cases in the study group [Figure 2a and b]. In the control group, moderate EGFR expression was seen in 60% and strong expression in 40% controls [Figure 3a and b]. This difference in EGFR expression was significantly different ($P < 0.001$) between study and control groups [Table 2].

Study group data were analyzed to find the association of tumor size, tumor grade, lymph node status, and FIGO stage with EGFR expression. Tumor size was significantly associated ($P < 0.001$) with the strong EGFR expression [Table 3]. We did not find any association of EGFR expression with histological grade, lymph node metastasis, and FIGO stage of the tumor [Tables 4-6].

Discussion

Cervical cancer being one of the most prevalent cancers among Indian females, is an important cause of morbidity and mortality among females.[20]

Many studies of EGFR in normal and dysplastic epithelium have shown contradictory results. Normally, EGFR is expressed in the basal cells of the ectocervical epithelium, and the intensity of expression may vary from mild to strong. As cells differentiate, the EGFR expression shifts from membranous to cytoplasmic, which is also evident in the present study. Soonthornthum *et al*. have shown that the intensity of EGFR expression in more in higher grades of CIN, and it is also associated with HPV infection.[21]

In the present study, we found that 3+ EGFR expression was present in significantly higher ($P < 0.001$) number of cases (96%) compared to controls. Kim *et al*.[22] compared EGFR expression between invasive cervical cancer and normal cervix found that invasive cervical cancer had significantly higher expression of EGFR as compared to control group. Li *et al*.[19] found that EGFR expression was present in the basal layer of the ectocervical epithelium. They also compared EGFR expression in SCC and CIN. They found that EGFR expression gradually increased from normal to low-grade to high-grade CIN and subsequently to invasive carcinoma. The difference in

Table 2: Comparison of epidermal growth factor receptor expression scores between cases and controls

EGFR expression	Cases	Controls	*P*[a]
Moderate (2+)	2	12	<0.001
Strong (3+)	28	8	

[a]Fischer's exact test. EGFR: Epidermal growth factor receptor

Table 3: Comparison of epidermal growth factor receptor expression with tumor size

EGFR expression	Tumor size (cm)		*P*[a]
	<4	>4	
Moderate (2+)	4	10	<0.03
Strong (3+)	0	16	

[a]Fisher's exact test. EGFR: Epidermal growth factor receptor

Table 4: Comparison of epidermal growth factor receptor expression with histological grade

EGFR expression	Histological grade			*P*
	Well	Moderate	Poor	
Moderate (2+)	0	4	0	0.31
Strong (3+)	4	16	6	

[a]Chi-square test. EGFR: Epidermal growth factor receptor

Table 5: Comparison of International Federation of Gynecology and Obstetrics stage with epidermal growth factor receptor expression

EGFR expression	FIGO stage			*P*[a]
	IB1	IB2	IIIB	
Moderate (2+)	2	0	0	0.39
Strong (3+)	14	4	10	

[a]Chi-square test. FIGO: International Federation of Gynecology and Obstetrics, EGFR: Epidermal growth factor receptor

Table 6: Comparison of epidermal growth factor receptor expression scores according to lymph node metastasis

EGFR expression	Lymph node metastasis		*P*[a]
	Present	Absent	
Moderate (2+)	0	2	0.30
Strong (3+)	10	18	

[a]Fischer's exact test. EGFR: Epidermal growth factor receptor

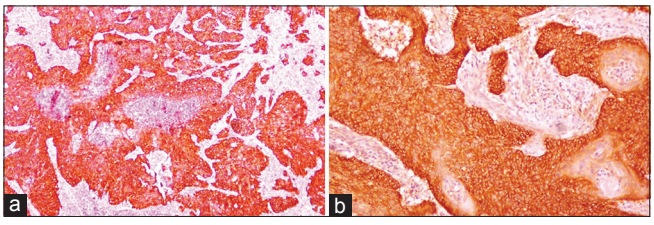

Figure 2: (a) Photomicrograph of moderately differentiated squamous cell carcinoma showing strong positivity with epidermal growth factor receptor (epidermal growth factor receptor, DAB, ×100), (b) Photomicrograph of moderately differentiated squamous cell carcinoma showing strong positivity with epidermal growth factor receptor (epidermal growth factor receptor, DAB, ×40)

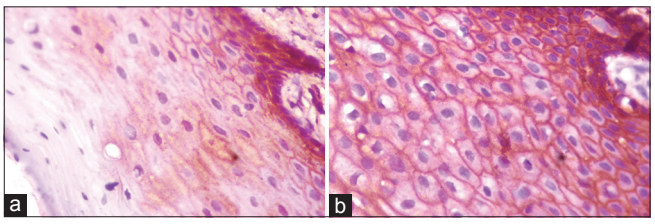

Figure 3: (a) Photomicrograph of chronic cervicitis showing moderate positivity with epidermal growth factor receptor (epidermal growth factor receptor, DAB, ×400), (b) Photomicrograph of chronic cervicitis showing strong positivity with epidermal growth factor receptor (epidermal growth factor receptor, DAB, ×400)

expression was not significant between high-grade CIN and invasive carcinoma. However, similar to our findings, the EGFR expression was significantly higher in cases with invasive SCC. Shen *et al*.[23] reported that EGFR overexpression (2+ or 3+) was found in 64% (35/53) of the primary cervical tumors. The difference in the findings may be explained by different methods and probably also different evaluation of the immunohistochemical staining.

We found that 26 out of 30 cases had tumor size >4 cm, out of these 16 cases showed 3+ EGFR expression, while 10 showed 2+, i.e., moderated expression. Tumor size was found to be associated with higher expression of EGFR ($P < 0.03$).

In the present study, EGFR expression score was higher in all grades of tumor (well, moderately, and poorly differentiated). Li *et al*.[19] compared degree of differentiation and EGFR expression and found that cases with both high and low degree of differentiation showed EGFR expression. Most cases, i.e., 5 out of 9 cases (55%) with higher differentiation and 9 out 15 cases (60%) with lower degree of differentiation has 2+–3+ EGFR immunoexpression. However, similar to our study, these differences were not statistically significant.

Out of 30 cases of SCC, all the cases of lymph node metastasis showed 3 + EGFR expression, while 90% cases without lymph node metastasis showed strong 3+ EGFR immunoexpression. Li *et al*.[19] found that out 19 cases with lymph node metastasis; three had 3+ and seven had 2+ EGFR expression, while four cases had 1+ and five cases negative immunostaining. Out of five node-negative cases, two cases have 3+ and 2+ and one case had 1+ expression. Shen *et al*.[23] found that 60% (32/53) of the cases with corresponding lymph node metastases showed 3+ EGFR expression. Similar to our study, in both the studies the EGFR expression was not associated with lymph node metastasis.

In the present study, we found no significant difference in EGFR expression between tumor of different clinical FIGO stages. Similar to our study, Kim *et al*.[22] also found that EGFR expression was not significantly different among various FIGO stages. Kristensen *et al*.[24] also did not find any association of tumor grade, stage, and lymph node status with EGFR expression.

The findings of the present study may be limited by its low sample size; however, it is suggested that a more extensive study taking a larger sample size might be needed to validate the findings of the present study.

Conclusions

The present study showed that a significantly higher number of cases of invasive SCC of uterine cervix show increased EGFR expression. The EGFR expression is associated with tumor size. The present study supports the thought that use of anti-EGFR monoclonal antibodies against certain subset of cervical SCC can act as a potential therapeutic option.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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