

Original Research Article**Expression of Epidermal Growth Factor Receptor (EGFR) in Cervical Intraepithelial Neoplasia and Squamous Cell Carcinoma of Uterine Cervix****Shaila Mitra¹, Rajesh Kumar Rai², Divya Rai³**¹Professor and Head ²Professor ³Resident 3rd year, Department of Pathology, B.R.D. Medical College, Gorakhpur, Uttar Pradesh 273013, India.**Abstract**

Background: Increased expression of the epidermal growth factor receptor (EGFR) gene has been shown in a large number of tumors, generally indicating a more aggressive biological behavior of cancers than those with low or normal expression. The role of EGFR in the tumorigenesis of the uterine cervix has been poorly understood and controversial.

Aim: In order to explore the relationship between EGFR status and cervical carcinoma this study has been conducted to evaluate the expression of epidermal growth factor receptor (EGFR) protein in benign conditions of cervix, cervical intraepithelial lesions(CIN) and squamous cell carcinoma(SCC) of cervix.

Material & Methods-: Immunohistochemical expression of EGFR in 106 cases of various lesions of uterine cervix was studied including chronic non specific cervicitis, CIN and SCC then correlation between histopathological diagnosis and EGFR immunostaining was also observed.

Results: Most of the cases of chronic cervicitis (87.5%) were negative for EGFR expression. 9/ 21 cases (42.9%) of low grade CIN showed positivity for EGFR while 27/36 (77.78%) of high grade CIN expressed EGFR. 26/33 cases (78.79%) of invasive squamous cell carcinoma showed strong immunoeexpression of EGFR. A gradual increase in the intensity and rate of expression from chronic cervicitis, low grade CIN and high grade CIN to invasive squamous cell carcinoma was observed.

Conclusion: A statistically significant correlation was found between increase of EGFR positivity from CIN to SCC. EGFR expression level in the premalignant.

Keywords: EGFR; CIN; SCC; Uterine Cervix.

Corresponding Author:

Shaila Mitra,
Professor and HOD,
Department of Pathology,
B.R.D. Medical College,
Gorakhpur, Uttar Pradesh 273013,
India.

E-mail:
shaila.prasad14@yahoo.co.in

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Introduction

Cervical cancer is most common cancer of female genital tract in India with approximately 1,00,000 new cases occurring each year. This accounts for almost 20% of all new cases diagnosed in the world annually [1]. It kills around 72,000 women in India every year, more than 26% of 2,7500 deaths worldwide. The mortality rate in India is 15.2% according to chart CCFC (cervical cancer free collation) compiled using data from WHO, the United Nations, the World Bank and the International Agency for Research on

Cancer Globocan [2]. Invasive cervical carcinoma is the end result of a long pathological process that begins with precursor lesions called cervical dysplasia and squamous intraepithelial lesion (SIL). CIN is not a cancer. Most cases of CIN remain stable, or are eliminated by the host's immune system without intervention. However, a small percentage of cases progress to become squamous cell carcinoma(SCC), if left untreated [3]. Many biological factors that help to regulate cell cycle control, apoptosis, angiogenesis, or invasive or metastatic potential have been proposed as prognostic determinants of cervical

cancer. Examples include epidermal growth factor receptor family (EGFR), vascular endothelial growth factor, microvessel density, hypoxic mechanism and expression of COX-2 [4].

EGFR is a 170-kDa transmembrane glycoprotein receptor encoded by Her-1 protooncogene located on chromosome 7p12. EGFR functions through dimerization that activates a tyrosine kinase domain to regulate multiple functions such as cell growth, differentiation, gene expression and development [4]. In normal cervical mucosa, EGFR is normally expressed in the cytoplasm and the membrane of cells within the basal layer, and as cell differentiate there is shift toward cytoplasm.

EGFR expression associated with HPV infection as EGFR cytoplasmic expression increases with increasing grade of intraepithelial neoplasia but is not correlated with HPV type [5]. In cervical cancer, the expression of EGFR varies depending on study methodology. EGFR showed negative expression in normal cervical tissue epithelia and low expression in epithelia of low grade CIN patients, but the ratio and intensity of expression in high grade CIN and cervical cancer increases gradually. It is also found that detection of EGFR expression can be used for early diagnosis and prognosis of cervical cancer [6].

Material and Methods

This cross-sectional study was conducted on patients coming to OPD and admitted in Obstetrics and Gynaecology wards of Baba Raghav Das Medical College, Gorakhpur on 106 cases of various lesions of cervix including benign lesions, cervical intraepithelial neoplasia (CIN) and squamous cell carcinoma (SCC) during a period of one year from July 2016 to July 2017. Patients of clinically suspected benign cases, cervical intraepithelial neoplasia (CIN) and invasive squamous cell carcinoma and patients who agreed to sign on consent form were included in the study. All autolysed and inadequate samples were excluded from the study.

Paraffin blocks of tissue were processed for 4 micrometer sections and stained with Hematoxylin & Eosin stain for morphological diagnosis after concordance of a double blind evaluation by two independent pathologists. Freshly cut sections were used for immunohistochemical detection of EGFR expression. Anti-EGFR monoclonal antibody (Clone 31G7) was obtained from BioSB, USA using a modified avidin-biotin immunoperoxidase method as described previously [7].

The reaction was considered positive when a brown color was seen in either cell membrane or cytoplasm. The sections were scored according to relative number of stained cells and the intensity of staining.

Two parameters were evaluated: relative number of stained cells & staining intensity. The present work has been conducted after getting ethical clearance from the institutional ethical committee.

Result

Out of total 106 cases studied, 16 (15.09%) cases were chronic non specific cervicitis, 21 (19.82%) cases were of low grade CIN, 36 (33.96%) of high grade CIN while invasive carcinoma were seen in 33 (31.13%) of cases. On analysis of immunohistochemical study, out of 16 cases of chronic cervicitis, only low expression of EGFR was observed in most of the cases. All these cells were present in the basal layer. Small amount of basal layer cells that showed positive staining were regarded as negative. Only 2 cases of chronic cervicitis (12.5%) showed weak EGFR expression (Figure 1).

Among CIN, 9 out of 21 cases (42.9%) showed positivity for EGFR immunopositivity. Expression was seen in spinous layers of the epithelium. 27 cases (77.78%) of high grade CIN expressed EGFR. From CIN I, expression of EGFR in the cells in the spinous layer began to appear & staining of cells in the mid surface gradually manifested in CIN II/ CIN III (Figure 2,3).

Out of 33 cases of invasive squamous cell carcinoma, 26 cases (78.79%) showed strong immunopositivity of EGFR. Cells present on the edge of the nest of tumour cells were more strongly stained than those in the middle. A gradual increase in the intensity and rate of expression from chronic cervicitis, low grade CIN and high grade CIN to invasive squamous cell carcinoma was observed. (Table 1; Figure 4)

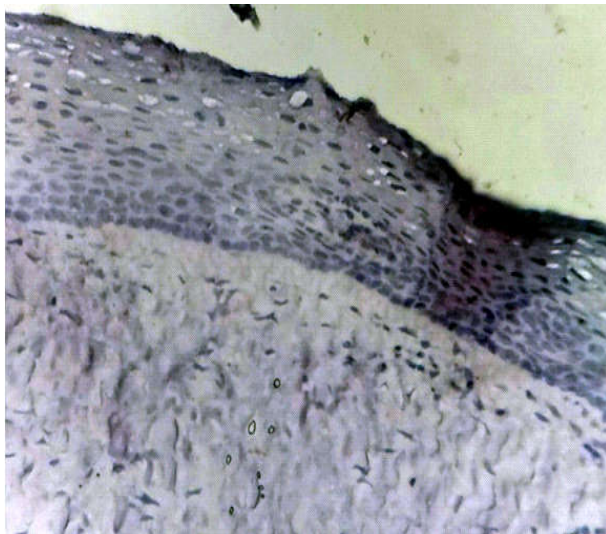
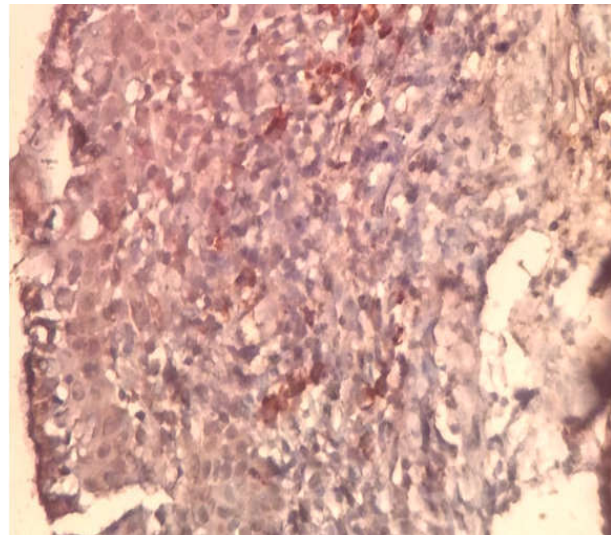
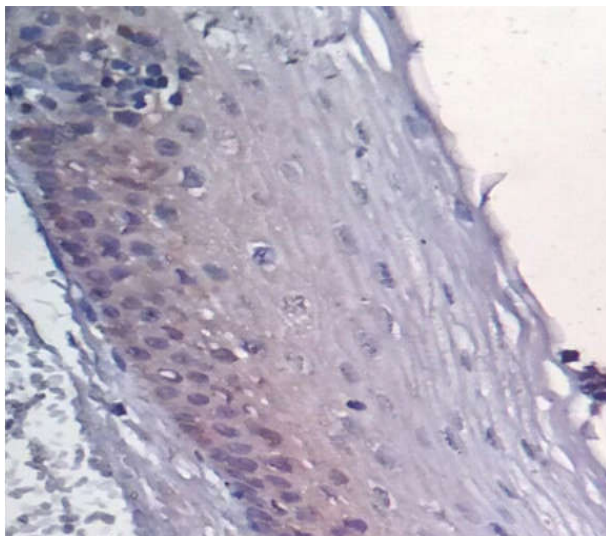
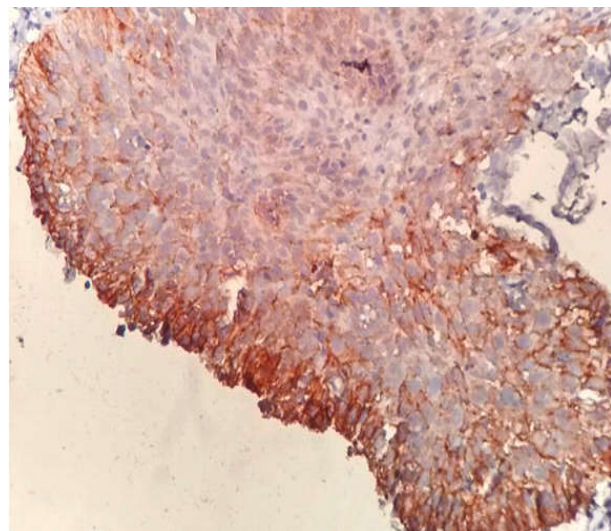
On analyzing the correlation of intensity of reaction of EGFR with chronic cervicitis, various CIN and invasive squamous cell carcinoma, out of 16 cases of chronic cervicitis, 14 cases (87.5%) were negative while 2 cases (12.5%) showed weak immunopositivity observed in basal cells. In cervical intraepithelial neoplasia (CIN), Among low grade CIN, the strong distribution was identified in basal, parabasal cells and in koilocytes, was moderate in 6 cases (66.66%) and weak in 3 cases (33.33%). In HSIL cases, the staining intensity was assessed as strong (+++) in 7 cases (25.93%), moderate (++) in 14 cases (51.85%) and weak in 6 cases (22.22%). Majority of invasive carcinoma cases (26/33) showed high expression of EGFR. Among them 61.54% (16/26) expressed moderate EGFR level whereas strong EGFR expression was observed in 38.46% (10/26) of cases. In latter cases, membranous & cytoplasmic staining occurred in more than 50% of cells. (Table 2).

Table 1: Expression of EGFR protein in various cervical lesions

| Histopathological diagnosis | Total No. of cases | Positive cases | Percentage (%) |
|-----------------------------|--------------------|----------------|----------------|
| Chronic cervicitis | 16 | 2 | 12.5% |
| Low grade CIN | 21 | 9 | 42.9% |
| High grade CIN | 36 | 27 | 77.78% |
| Cervical cancer | 33 | 26 | 78.79% |
| Total | 106 | 64 | 100% |

Table 2: Correlation between histopathological diagnosis and egfr immunostaining

| Histopathological diagnosis | -ve No. (%) | Weak positive (+) No. (%) | Moderately positive (++) No. (%) | Strong positive (+++ No. (%) |
|--------------------------------------|-------------|------------------------------|-------------------------------------|---------------------------------|
| Chronic non specific cervicitis (16) | 14 (87.5%) | 02 (12.50%) | 00 (00.00%) | 00 (00.00%) |
| Low Grade CIN (21) | 12 (57.14%) | 03 (33.33%) | 06 (66.66%) | 00 (00.00%) |
| High Grade CIN (36) | 09 (25.00%) | 06 (22.22%) | 14 (51.85%) | 07 (25.93%) |
| SCC (33) | 07 (21.21%) | 00 (00.00%) | 16 (61.54%) | 10 (38.46%) |
| Total=106 | 42 | 11 | 36 | 17 |

**Fig. 1:** Microphotograph showing negative EGFR expression Chronic cervicitis (x100)**Fig. 3:** CIN 3: EGFR staining shows strong diffuse positivity of cytoplasm & cytoplasmic membrane. (x400)**Fig. 2:** CIN I: EGFR immunostaining demonstrates positivity in cytoplasm of lower 1/3 of cells in epithelium. (x100)**Fig. 4:** Microphotograph of invasive (Non keratinizing) squamous cell carcinoma showing strong EGFR positivity (x100)

The difference of EGFR expression between high grade CIN and SCC was found to be statistically insignificant, ($p < 0.931$). Difference of EGFR expression between SCC and low grade CIN was statistically significant. ($p < 0.02$), between squamous cell carcinoma (SCC), benign lesion (chronic cervicitis) was found to be statistically significant ($p < 0.0001$). The rate of EGFR expression in the high grade CIN group was higher than in the low grade ($p < 0.032$) and benign group ($p < 0.0001$) and the difference was significant and highly significant respectively.

Discussion

Cervical cancer is third largest cause of cancer mortality in India after cancers of oral cavity, and esophagus and it accounts for almost 20% of all new cases diagnosed in the world annually. The proposed study was conducted on patients coming to OPD and admitted in Obstetrics and Gynaecology wards of Baba Ragahav das Medical College, Gorakhpur. Study has been conducted on 106 cases on women ages 21-70 years presented with benign lesions, cervical intraepithelial neoplasia (CIN) and squamous cell carcinoma (SCC). On the basis of histopathological examination, out of total 106 cases taken in the study 16 cases (15.09%) were chronic cervicitis, 21 cases (19.82%) were classified as low grade CIN, 36 (33.96%) as high grade CIN and 33 (31.13%) as invasive carcinoma. Similar finding was reported by *Quing Li et al (2014)*⁸ in which out of 75 cases 10 (13.33%) patients had chronic cervicitis, 16 (21.33%)

had low grade cervical intraepithelial lesion (CIN), 25 (33.33%) had high grade CIN and 24 (32%) had cervical cancer.

By immunohistochemistry, EGFR showed only low expression in non malignant cervical (chronic cervicitis) squamous epithelial cells. All these cells were present in the basal layer (such cells were used for inherent controls). Small amounts of basal layer cells that showed positive staining were regarded as negative. From CIN level I, expression of EGFR in the cells in the spinous layer began to appear, and staining of cells in the mid surface gradually manifested in CIN-II/ CIN-III. Cells on the edge of the cancer nest were more strongly stained than those in the middle. In the development of the lesion at all levels from chronic cervicitis, low grade CIN and high grade CIN to squamous cell carcinoma, the rate of expression increased gradually and the intensity also increased gradually. With the help of immunohistochemistry its expression was observed in the plasma membrane and cytoplasm in all cervical lesions.

On analyzing the expression of EGFR among various CIN group, the results of present study are concordant with the results of previous studies. In our study 42.9% of LSIL while 77.78% of HSIL showed positivity for EGFR respectively. The results of present study are in concordance with the study conducted by *Balan R et al (2011)* [9] and *Quing Li et. al. (2014)* [8]. (Table 3)

In our study, 78.79% of the cases of invasive carcinoma were EGFR positive. Our findings are in concordance with

Table 3: Comparison of various studies on cin using egfr protein expression

| Lesions | Present study | Balan R et al(2011) ⁸ | Quing Li et al(2014) ⁷ |
|---------|---------------|----------------------------------|-----------------------------------|
| LSIL | 9/21 (42.9%) | 16(32%) | 7(43.75%) |
| HSIL | 27/36(77.78%) | 33(67%) | 19(79.17%) |

Table 4: Comparison of various studies on invasive carcinoma using egfr protein expression

| Author | Invasive carcinoma |
|---------------------------------------|--------------------|
| Present study | 26/33(78.79%) |
| Kim JW et al(1996) ¹⁰ | 29/40(72.5%) |
| El Hamdani et al (2010) ¹¹ | 47/53(88.67%) |
| Viswanath Let al(2014) ¹² | 71/78(91.02%) |
| Quing Li et al(2014) ⁸ | 19/24(79.17%) |

Kim J W et. al. (1996) [10] and Quing Li et. al. (2014) [8] whose study showed EGFR positivity 72.5% and 79.17% respectively.

In our study, regarding LSIL category, the staining distribution was identified in basal, parabasal cells and koilocytes and was considered strong in 00.00%, moderate in 6 (66.66%) and weak in 3 (33.33%) cases. In HSIL cases, the staining was strong in 07(25.93%), moderate in 14 (51.85%) and weak in 06 (22.22%) cases. We have compared

our study with Raluca Balan et. al. (2011) [9] where in LSIL category, the staining intensity was strong in 10 (10%), moderate in 21 (64%) cases and weak in 8(26%) cases. In HSIL category, strong positivity in 15 (87%) cases, moderate in 1 (7%) case and weak in 2 (6%) cases.

In present study, regarding invasive squamous cell carcinoma Majority of invasive carcinoma cases (26/33) showed high expression of EGFR. Among them 61.54% (16/26) expressed moderate EGFR level whereas strong

EGFR expression was observed in 38.46% (10/26) of cases. In latter cases, membranous & cytoplasmic staining occurred in more than 50% of cells. Our study in concordance with Hamdani El et. al. (2010) [11], who studied 47 cases of invasive squamous cell carcinoma of cervix, where 29/47 cases (61.7%) shows moderate EGFR levels whereas strong EGFR expression was observed in 18/47 cases (38.2%) (Table 4).

On statistical evaluation using Chi-square test, the rate of EGFR expression in squamous carcinoma cells was higher than high grade CIN cells, but not statistically significant ($p=0.931$). However, EGFR expression in squamous cell carcinoma was higher than low grade CIN and chronic cervicitis and these differences were significant ($p=0.02$; $p=0.0001$). The rate of EGFR expression in the low grade CIN was higher than chronic cervicitis group but it showed no statistical significant differences ($p=0.1013$).

There is very little data, regarding the sensitivity and specificity of EGFR in cervical intraepithelial neoplasia and squamous cell carcinoma is currently available in the medical literature. Grass B et. al. [13] evaluated sensitivity and specificity of EGFR in rhabdomyosarcoma and found 93.2% and 74.1% respectively. Ganti et. al. [14] and Wachter et. al. [15] found sensitivity 76% and 84% respectively and specificity 84% and 80% respectively.

Conclusion

EGFR showed negative expression in normal cervical tissue epithelia and low expression in epithelia of low grade CIN patients, but the ratio and intensity of expression in high grade CIN and cervical cancer increases gradually. A statistically significant correlation was found between increase of EGFR positivity from CIN to SCC. EGFR expression level in the premalignant lesion appears to be a sensitive factor in predicting the neoplastic potential of dysplastic tissue. This suggests that EGFR may serve as a biological marker to identify high risk subgroups and can be useful as target for new treatment modalities. However, further studies are needed to evaluate the clinical utility of EGFR expression as a tumour marker in cervical carcinogenesis.

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