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# The role of epidermal growth factor receptor as progression factor in cervical intraepithelial neoplasia and squamous cell carcinoma

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## ABSTRACT

**Background:** Cervical carcinoma is the most common cancer with a prevalence of 28.6% of all cancers in women in Indonesia and is one of the leading causes of death among gynecologic malignancies. Prevention and understanding are needed in terms of initiation and development of precancerous lesions into cervical cancer. About 90% of cervical cancers are squamous cell carcinomas that develop from intraepithelial neoplasia. Epidermal Growth Factor Receptor (EGFR) is an expression product of the proto-oncogene c-erbB-1 (HER-1), which plays a role in the proliferation, differentiation and acceleration of the transformation of malignant cells. This study aimed to prove the role of EGFR as a progression factor of cervical intraepithelial neoplasia and cervical squamous cell carcinoma.

**Methods:** The study design was a cross-sectional analytic observational study, with a total sample of 36, which was taken from biopsy or surgery from patients with cervical intraepithelial neoplasia (CIN) and cervical invasive squamous cell carcinoma (SCC), whose tissues were examined at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Udayana/Sanglah General Hospital. Histopathological diagnosis and determination of the type of malignancy were carried out by staining with Hematoxylin and Eosin (H & E). The immunohistochemical stain evaluated EGFR expression.

**Results:** The age range of CIN was 21-48 years and SSC 33-61 years, mean age 41.8 ± 10.55 years. A total of 16 (44.4%) showed CIN namely 9 (25%) low-grade CIN, 7 (19.4%) high-grade CIN, and 20 (55.6%) SCC. There was a significant difference in the expression of EGFR at low-grade CIN, high-grade CIN and SCC ($p = 0.004$). There was a significant difference in EGFR expression between low-grade CIN and high-grade CIN ($p = 0.035$) and between low-grade CIN and SCC ($p = 0.003$). There was no significant difference in EGFR expression between high-grade CIN and SCC ($p = 0.441$).

**Conclusion:** EGFR has a role as a progression factor in CIN and SCC. There was no difference in the expression of EGFR between high-grade CIN and SCC, probably because in this study, carcinoma in situ was also included in high-grade CIN.

**Keywords:** cervical intraepithelial neoplasia, EGFR, squamous cell carcinoma.
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## INTRODUCTION

Cervical cancer is a malignancy in women that ranks second globally and accounts for 15% of all cancers in women, with a prevalence of about 500,000 new cases and 275,000 morbidities each year. About 80% of these cases occur in developing countries, and > 80% were diagnosed at an advanced stage.[1,2] Efforts are needed to prevent and understand the onset and progression of precancerous lesions to cervical cancer. About 90% of cervical cancers are squamous cell carcinomas that develop from cervical intraepithelial neoplasia (CIN). The progression from early infection by high-risk human papillomavirus (HPV) to intraepithelial neoplasia and cervical carcinoma takes years or even decades.[3] Deep knowledge about predictive biomarkers is needed to be used in prevention and treatment considerations.[4]

Epidermal Growth Factor Receptor (EGFR) is a 170-kDa-heavy transmembrane glycoprotein receptor encoded by the proto-oncogene Her-1 chromosome 7p12. The function of EGFR is activated through dimerization by activating the tyrosine kinase domain to regulate various functions such as cell growth, differentiation, gene expression, and acceleration of malignant cell transformation.[1,4] In normal cervical mucosa, EGFR is expressed in the cytoplasm and cell membrane of basal cells. After the cells are differentiated, EGFR is expressed in the cytoplasm, where its expression increases with increasing degrees of intraepithelial neoplasia and cervical squamous cell carcinoma.[1] This study aimed to prove the role of

EGFR as a progression factor of cervical intraepithelial neoplasia (CIN) and invasive cervical squamous cell carcinoma (SCC).

## RESEARCH DESIGN AND METHODS

### *Specimen collection*

The study design was a cross-sectional analytic observational study that was conducted during the year 2020, with a total sample of 36 which was taken from biopsy or surgery from patients with cervical intraepithelial neoplasia and cervical invasive squamous cell carcinoma, whose tissues were examined at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Udayana/Sanglah General Hospital, which correspond to inclusion and exclusion criteria. New patients and never received chemotherapy or radiotherapy were included in this study, and the specimens containing a lot of necrotic and hemorrhaged tissue and damaged paraffin blocks were excluded. Samples were collected on a consecutive basis until the required sample size was met according to the sample's calculation. This study has been permitted by the ethical committee of the faculty of medicine, Universitas Udayana, with letter number 1013/UN14.2.2.VII.14/LT/2020.

### *Histopathological and Immunohistochemical of EGFR Examination*

This study was conducted at Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Udayana/Sanglah General Hospital. Histopathological diagnosis of cervical intraepithelial neoplasia and the determination of the degree of low or high, and determination of invasive squamous cell carcinoma were carried out by staining specimens with Hematoxylin Eosin (H & E).

The paraffin-embedded tissues from biopsy or surgery tissue of patients that have been diagnosed histopathologically with low-grade and high-grade cervical intraepithelial neoplasia and invasive squamous cell carcinoma were examined immunohistochemically to evaluated EGFR expression. The Cell Marque Rabbit Monoclonal Antibody EGFR (SP 84) Vantagebio was used.

**Table 1. Sample Distribution Based on Age Range**

| Age Range (years) | Low-grade CIN | High-grade CIN | SCC | Total |
|---|---|---|---|---|
| | n (%) | n (%) | n (%) | n (%) |
| 21-30 | 4 (44.4) | 2 (28.6) | 0 ( 0.0) | 6 (16.7) |
| 31-40 | 4 (44.4) | 3 (42.8) | 6 (30.0) | 13 (36.1) |
| 41-50 | 1 (11.2) | 2 (28.6) | 6 (30.0) | 9 (25.0) |
| 51-60 | 0 ( 0.0) | 0 ( 0.0) | 7 (35.0) | 7 (19.4) |
| 61-70 | 0 ( 0.0) | 0 ( 0.0) | 1 ( 5.0) | 1 ( 2.8) |
| Total | 9 (100) | 7 (100) | 20 (100) | 36 (100) |
| Mean ± SD 41.8 ± 10.55 | | | | |

**Table 2. Sample Distribution Based on Grade of CIN and Invasive Squamous Cell Carcinoma**

| Grade of CIN dan SCC | Number | |
|---|---|---|
| | n | % |
| Low-grade CIN | 9 | 25.0 |
| High-grade CIN | 7 | 19.4 |
| Subtotal | 16 | 44.4 |
| SCC | 20 | 55.6 |
| Total | 36 | 100 |

**Table 3. Sample Distribution Based on EGFR Expression and Grade of CIN and Invasive Squamous Cell Carcinoma**

| EGFR Expression | Grade of CIN | | SCC | Total | p |
|---|---|---|---|---|---|
| | Low-grade n (%) | High-grade n (%) | n (%) | n (%) | |
| Low | 9 (42.9) | 4 (19.0) | 8 ( 38.1) | 21 (58.3) | 0.004 |
| High | 0 (00.0) | 3 (20.0) | 12 (80.0) | 15 (41.7) | |

**Table 4. Grade of CIN and Invasive Squamous Cell CarcinomaBased on the Result of the Mann-Whitney Test**

| Grade of CIN and SCC | p |
|---|---|
| Low-grade CIN<br>High-grade CIN | 0.035 |
| Low-grade CIN<br>SCC | 0.003 |
| High-grade CIN<br>SCC | 0.441 |

### *Statistical Analysis*

A descriptive characteristic of the data subject was tabulated. The Shapiro-Wilk test was performed to determine the normality of the data. The Kruskal Wallis test was performed to evaluate the differences in EGFR expression in low-grade CIN, high-grade CIN and cervical invasive squamous cell carcinoma, and followed by Post Hoc because the data are not normally distributed. Statistical significance for this test was set at 2-sided of 0.05 levels with 95% confidence interval (CIs), p-values smaller than 0.05 were considered statistically significant.

## RESULTS

The study results are shown in Table 1 to Table 4, which shows the distribution of samples based on age, grade of cervical

intraepithelial neoplasia (CIN) and invasive squamous cell carcinoma (SCC), and EGFR expression. Table 1 shows the distribution of samples based on the range of age.

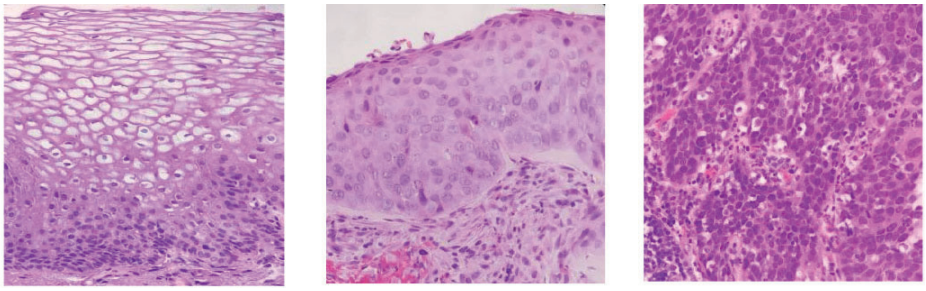
It was found that the age range of CIN was 21-48 years, while that of SCC was between 33-61 years. The largest distribution was found in the 31-40 years age group, namely 13 samples (36.1%), with a mean age of 41.8 ± 10.55 years. At low-grade CIN, the highest distribution was found in the 21-30 years and 31-40 years age group, respectively, with 4 (44.4%) samples. At the high-grade CIN, the most distribution was found in the 31-40 year age group of 3 (42.8%) samples. In SCC, the most distribution was found in the age group 51-60 years with 7 (35.0%) samples.

Based on the degree of CIN and SCC, 16 (44.4%) showed precancerous lesions (CIN), consisting of 9 (25%) samples showing low-grade CIN, 7 (19.4%) samples high-grade CIN, while SCC was 20 (55.6%) samples.

Low EGFR expression was obtained in all samples of low-grade CIN, namely nine samples (42.9%), 4 (19.0%) in high-grade CIN, and 8 (38.1%) in invasive SCC. There was no high-EGFR expression at low-grade CIN, while high-EGFR expression was found at high-grade CIN as many as 3 (20.0%) and 12 (80.0%) invasive SCC samples. The most low-EGFR expression was found at low-grade CIN. The most high-EGFR expression was found in invasive SCC. Data normality test was carried out with Shapiro-Wilk Test, obtained $p = 0.008$ ($p < \alpha$), the data was not normally distributed. Kruskal-Wallis Test was performed, and it was a significant difference in EGFR expression at low-grade CIN, high-grade CIN and SCC ($p = 0.004$).

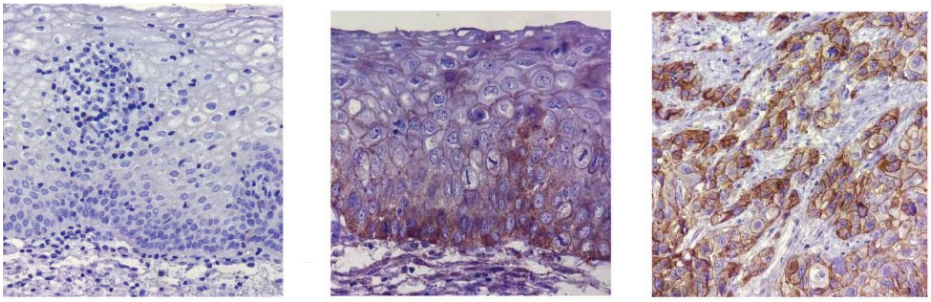
Mann-Whitney test was performed to determine the differences in expressions between variables. There was a significant difference in EGFR expression between low-grade CIN and high-grade CIN ($p = 0.035$), also between low-grade CIN and SCC ($p = 0.003$). There was no significant difference in EGFR expression between high-grade CIN and SCC ($p = 0.441$).

Study samples with H & E staining according to the CIN and invasive SCC grade are shown in Figure 1. The results of the EGFR immunohistochemical (IHC) stain are shown in Figure 2.



A B C

**Figure 1.** CIN and Invasive SCC, A. Low-grade CIN (H & E, 40x), B. High-grade CIN (H & E, 100x), C. Invasive SCC (H & E, 100x)



A B C

**Figure 2.** EGFR Expression in CIN and Invasive SCC, A. Low-grade CIN, low expression (IHC, 40x), B. High-grade CIN, high expression (IHC, 100x), C. Invasive SCC, high expression (IHC, 400x)

## DISCUSSION

The primary cause of precancerous lesions and cervical cancer is infection with high-risk HPV, especially types 16 and 18. The infection is usually transmitted through sexual contact and causes precancerous lesions or cervical intraepithelial neoplasia. Most of the lesions usually disappear spontaneously after 6-12 months due to immunological processes. A small proportion of these lesions will persist and can cause cancer.[2,5–7] Cervical cancer often originates in the transforming region and develops slowly in the human body.[8] With earlier screening and treatment of precancerous lesions, progression to cancer is prevented.[2]

In patients with precancerous lesions, research conducted by Modinou et al. (2011) found the mean patient age was 37.03 ± 10.47 years. In young women (17-30 years), no one has high-grade CIN, low-grade CIN (CIN 1) occurs in women with an older age group (30-35 years), and high-grade CIN (CIN 3) occurs in the even older age group (36-50 years).[2] In this study, it was found that the age range of CIN was between 21-48 years, while SCC was between 33-61 years. The largest distribution was found in the 31-40 years age group, namely 13 samples (36.1%), with a mean age of 41.8 ± 10.55 years. At low-grade CIN, the most distribution was found in the 21-30 years and 31-40 years age group, respectively, with 4 (44.4%) samples. At high-grade CIN, the most distribution was found in the 31-40 years age group, namely 3 (42.8%) samples, and in SCC, the most distribution was found in the age group 51-60 years with 7 (35.0%) samples. With increasing age, the progression to high-grade lesions and SCC also increased.

Research conducted on patients with precancerous lesions showed the incidence of precancerous lesions was 49.5%, with a low-grade CIN of 16.7% and a high-grade CIN of 83.3%.[2] In this study, 16 (44.4%) samples showed precancerous lesions

(CIN), consisting of 9 (25%) samples showing low-grade CIN, 7 (19.4%) samples of high-grade CIN, while SCC of 20 (55,6%) sample.

The permanent high-risk human papillomavirus (HPV) infection is a causative agent for cervical cancer, but in order to progress from CIN, changes in oncogenes and tumor-suppressor genes are required.[5,9–11] The main mechanism by which HPV plays a role in cervical cancer carcinogenesis involves two viral oncoproteins, E6 and E7 and their interactions with the tumor-suppressor genes P53 and Rb, which will affect genetic integrity, cell adhesion, immune response, apoptosis and cellular control.[5] The combination of various biological, socioeconomic, and health factors also play a role as a risk factor for cervical cancer, such as infections of the cervix that are transmitted through sexual contact, reproductive factors, sexual intercourse at a young age, hormonal, genetic, smoking, multiple sexual partners and parity.[5,8]

Previous studies have shown that EGFR expression is associated with HPV infection but not with HPV type.[1,9] The expression of EGFR increases with increasing degrees of CIN. Protein E5 from HPV type 16 can activate EGFR by binding with a subunit of the ATPase protein pump, causing decreased EGFR receptor degradation, increased EGFR cycle and EGFR overexpression. Expression of E6 is also associated with increased levels of EGFR. Changes in functional levels of E6/E7 protein can change the growth rate of cervical cancer by decreasing EGFR stability. The high-risk HPV protein affects the EGFR protein but not at the genomic level.[1]

DNA amplification in certain chromosomal regions is one of the mechanisms of gene activation for the development and progression of cancer. In SCC, several proto-oncogenes often undergo activation using amplification, including EGFR (7q12), MYC (8q24), HRAS (11q15.5), ERBB2 (17q11.2-12), CCND1 (11q13), and cIAP1 (11q22). EGFR amplification is obtained in 10% of cases of SCC.[9] Cytogenetic and molecular biology studies have shown that the majority of solid tumors exhibit chromosomal instability. In cervical cancer, this instability occurs on chromosomes 3, 8, 5, 7, X, and 18, which occurs at the beginning of carcinogenesis.[4]

EGFR is a 170-kDa transmembrane glycoprotein receptor, a member of the ErbB family of tyrosine kinase receptors that function to transmit signals that induce cell growth, namely proliferation or growth, differentiation, invasion, migration, neovascularization and accelerate the transformation of malignant cells, located on chromosome 7 p13-7 p12. ErbB family members consist of 4 receptors, namely: ErbB-1 (EGFR or HER-1), ErbB-2 (HER2 or Neu), ErbB-3, and ErbB-4.[1,4,9,12,13]

EGFR is expressed on various normal tissues and solid tumors. Normal EGFR is expressed on the cytoplasm and cell membrane in the basal cell layer. After the cell is differentiated, EGFR expression moves from the cell membrane into the cytoplasm.[1,13] Research conducted by Li et al. (2014) found an increase in the value and intensity of EGFR expression ranging from chronic cervicitis, low-grade CIN, high-grade CIN and SCC. EGFR expression in SCC was higher than that of high-grade CIN, but it was not statistically significant. The difference in expression between SCC, low-grade CIN and chronic cervicitis was significant, as well as the expression of EGFR in high-grade CIN was higher than that of low-grade CIN and chronic cervicitis and was statistically significant.[4] Research by Yadav et al. (2019) who examined EGFR expression in SCC and its relationship with tumor characteristics, found strong EGFR expression in 93.4% of cases, and moderate expression in 6.6% of cases. EGFR expression was significantly higher in invasive SCC.[13]

In this study, low EGFR expression was obtained in all low-grade CIN samples, namely 9 (42.9%), 4 (19.0%) high-grade CIN and 8 (38.1%) invasive SCC. There was no high EGFR expression at low-grade CIN, and a high EGFR expression was obtained at high-grade CIN as many as 3 (20.0%) and 12 (80.0%) invasive SCC. Most of the low-EGFR expression was found at low-grade CIN. The most high-EGFR expression was found in invasive SCC. There was a significant difference in EGFR expression at low-grade CIN, high-grade CIN and SCC ($p = 0.004$). Mann-Whitney test showed a significant difference in EGFR expression between low-grade CIN and high-grade CIN ($p = 0.035$), also a significant difference in EGFR expression between low-grade CIN and SCC ($p = 0.003$), but there was no significant difference in EGFR expression between high-grade CIN and SCC ($p = 0.441$).

Cases with high gene amplification showed moderate to strong EGFR protein expression, suggesting that EGFR gene amplification may be one of the molecular mechanisms underlying EGFR overexpression. That suggested that high levels of EGFR protein may be involved in cervical cancer progression.[4,9] Detection of EGFR protein at various degrees of CIN and SCC, gene amplification and variation in the number of copies of the gene can be used to distinguish low and high- grade NIS, and as an early diagnosis of cervical cancer, as well as an indicator of early prognosis.[4] Anti-EGFR therapy may be of benefit to patients who have 7p11.2 amplification of their tumor.[9]

EGFR overexpression is associated with poor prognostic factors, such as increased tumor size > 4 cm, lymph node metastases, FIGO stage III and IV, age ≥ 60 years.[1,14] Other studies found insignificant results. It could be perhaps due to differences in methodological protocols, antibody source and dilution, assessment of positive category from immunohistochemical results, and sample size.[9,13]

In this study, the sample size in accordance with the calculation of the sample size formula is 36, which may be used as a preliminary study for further studies with a larger sample size. It is also necessary to consider separating the in situ carcinoma from high-grade CIN group.

## CONCLUSION

EGFR has a role as a progression factor in CIN and SCC. There was no difference in the expression of EGFR between high-grade CIN and SCC, probably because in this study, carcinoma in situ was also included in high-grade CIN.

## DISCLOSURES

### Author Contribution

All authors have contributed to all processes in this research, including preparation, data gathering and analysis, drafting and approval for publication of this manuscript.

### Conflict of Interest

The authors declare no conflict of interest regarding the publication of this article.

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