

## RESEARCH ARTICLE

# Correlation between EGFR Expression and Radiosensitivity in Cervical Adenocarcinoma Cases

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### Abstract

**Background:** Most of the cervical cancer patients, including those with cervical adenocarcinomas, come at advanced stage in the developing world so its mortality is high. Radiotherapy is one of the treatment modality for advanced stage cervical adenocarcinomas, but its efficacy depends on several prognostic factors such as the stage, histopathology, presence of organ dysfunction and expression of cellular biology markers mainly involve in cell proliferation such as the epidermal growth factor receptor (EGFR). Some research indicates that activation of EGFR in malignancy (including cervical cancer) correlates with aggressive behavior, a poor prognosis and decreasing sensitivity of radiotherapy. However, the combination between targeted therapies and radiotherapy are innovative approaches which may provide a good result. This study aimed to assess any correlation between expression of EGFR and response to radiotherapy in cervical adenocarcinoma cases. **Materials and Methods:** A total of 32 women were registered in a retrospective study period January 2007 and May 2014. Paraffin blocks from these patients were processed by classical histological techniques and for immunohistochemical staining of EGFR, scoring being accomplished according to the immunoreactive scoring (IRS) of Remmele and Stegner. **Results:** Among the studied molecular factors, there was significant correlation expression of EGFR with poor response to radiotherapy ( $p=0.0001$ ). **Conclusions:** The result of this study showed a significant correlation between expression of EGFR and sensitivity of radiation in cervical adenocarcinoma cases. Further research is necessary to obtain information about new therapeutic management.

**Keywords:** Biological markers - cervical adenocarcinoma - radiotherapy - radiosensitivity

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### Introduction

Adenocarcinoma cervix is the second most common type of histopathology in cervical carcinoma cases (Wells et al., 2014). Cervical cancer (including cervical adenocarcinoma) has been the major health problems among Indonesian women because of its high incidence and mortality. Mostly of the diagnosed cases in Indonesia have been stated in advanced stages (IIB-IVB) (Imam, 2009). Radiotherapy is one of the modality treatment for advanced stage in cervical adenocarcinoma (Noordhuis et al., 2011; Wells et al., 2014). The effectiveness of radiotherapy depends on several prognostic factors such as the staging, histopathology of tumor, presence of organ dysfunction and cellular biology marker especially markers involved in cell proliferation such as epidermal growth factor receptor (EGFR) (Noordhuis et al., 2011, Gadducci et al., 2013).

In certain studies, the expression of the receptor for epidermal growth factor evident to be an independent predictor of worse prognosis for the cervical cancer, including cervical adenocarcinoma (Noordhuis et al., 2011, Barbu et al., 2013). Prohibiting EGFR through radiotherapy shall be a promising therapeutic strategy in

cervical cancer (Barbu et al., 2013).

The objective of this study was to search the coherency EGFR biomarkers in patients with advanced cervical adenocarcinoma and its response to radiotherapy. In addition, this study aims to quarry a notioning of the molecular mechanisms and to validate predictors in cervical adenocarcinoma treated with radiotherapy.

### Materials and Methods

This research has ethical clearance from Health Research Ethics Committee with number 256/UN6.C2.1.2/KEPK/PN/2014 on June 26<sup>th</sup>, 2014. This study use analytical observational technique and correlational study with retrospective cross-sectional design.

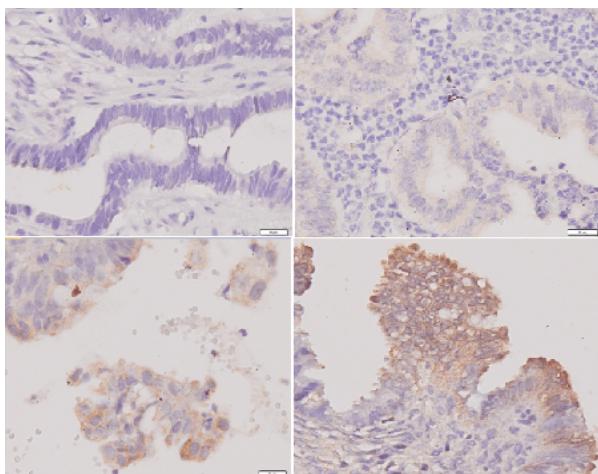
During January 2007 - May 2014, 32 women from admission were included in this study. Cervical tumor biopsies were taken before radiotherapy and diagnosed as cervical adenocarcinoma. Most of the patients with an age range of 41-59 years old. All of the patients have been received radical radiotherapy which were given based on the standard techniques and dosage in Oncology Radiation Department. Response of radiation for patients with radiotherapy was assessing later after finished

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**Table 1. Correlation between EGFR and Tumor Response**

	Response to Radiotherapy		Total	R	Statistic
EGFR	Complete	Not Complete			
Negative	13 (86.7%)	1 (5.9%)	14	0.800	p=0.0001**
Positive	2 (13.3%)	16 (94.1%)	18		
Total	15 (100%)	17 (100%)	32		

Noted : nominal vs nominal data, p value counted according Lambda correlation test.; Significantly value according p value <0,05 . \*\* means statistically significant. R means coefficient correlation



**Figure 1. Cervical Adenocarcinoma.** (A) EGFR negative reaction in cervical adenocarcinoma, 400x; (B) EGFR mild positive reaction in cervical adenocarcinoma, 400x; (C) EGFR moderate positive reaction in cervical adenocarcinoma, 400x; (D) EGFR strong positive reaction in cervical adenocarcinoma, 400x

radiotherapy and they were divided into two groups: complete response and incomplete response (partial response, stable disease and progressive response).

Paraffin blocks from these patients were processed by classical histological techniques with haematoxylin, eosin and immunohistochemical staining of EGFR. The immunohistochemical technique was applied on tissue samples fixed in 10% buffered formalin, paraffin included, and sectioned at 3–4 µm. The primary antibodies were used at dilution 1:100 for EGFR (clone H11, DAKO). Positive control for EGFR was used normal skin tissue or squamous cell carcinoma tissue (DAKO guidelines).

EGFR immunostaining assessment : regions of greatest immunoreactive cells was selected (at least five areas at a magnification of 400×) and the percentage of immunoreactive cells and intensity of immunoreactive cells were scored according to the immunoreactive scoring (IRS) by Remmele W and Stegner HE. (Barbu et al., 2013) Assessment EGFR staining, the percentage of immunoreactive tumor cells was rated as follows: no staining = 0;>0 -20% = 1 ; 21–50% = 2; 50–85% = 3; >85% = 4; intensity: weak (1+); moderate (2+); strong (3+). The grade of score expression was obtained by multiplying percentage with intensity. Score 0 = negative; 1–5 = weak; 5–8 = moderate; 9–12 = strong. (Marcela et al., 2012; Barbu et al., 2013)

The data obtained are recorded in a special form and processed through SPSS version 21.0 for Windows.

## Results

The data in this study including age, staging and grading histopathology. From the 32 patients with cervical adenocarcinoma, most of the patients were stage IIB and above (26 patients) at age range 41-59 years old as many as 21 patients. Most of them were diagnosed as high grade cervical adenocarcinoma.

The statistical analysis of this study was the analysis of Lambda correlation. Analyzing data for the correlation between the biomarkers EGFR and response to radiotherapy were obtained for EGFR with R = 0.800 and p = 0.0001. This study shows EGFR highly expressed in patients with incomplete response to radiotherapy. EGFR expression was correlating with sensitivity of radiotherapy (Table 1).

## Discussion

In this study, most of the cases in the range from 41 to 59 years old were 8 cases and over 60 years old were 3 cases. Cervical adenocarcinoma patients who used in this study, 6 cases were in stage IIA, and 26 cases were in stage IIB and above. The proportion of adenocarcinoma of the cervix uteri in General Hospital Hasan Sadikin Bandung period January 2009 - December 2011 between 8% -12%. Majority patients with cervical carcinoma including adenocarcinoma of the cervix were admitted in advanced stage, so the diagnosis and management has been delayed because Indonesian society is lack of self-awareness to do the screening especially rural communities and it will increase mortality rate. (Imam R., 2009) Cervical cancer including cervical adenocarcinoma has been afflicted a number of women, but while it has become prevalent, it may be cured if detected early. Women reproductive aged and sexually active and those who have a family history of cancer are encourage to undergo screening. (Pimenta et al., 2013)

At this time in our institution seeks to obtain new therapeutic strategies for cervical adenocarcinoma patients. The experts found worse prognosis of cervical adenocarcinoma than cervical squamous cell carcinoma, particularly in patients with positive lymph nodes with a shorter recurrence interval. (Kato et al., 2013) The medication of patients with advanced cervical adenocarcinoma by incorporating neo-adjuvant chemotherapy and chemoradiation is highly effective. (Tang J et al., 2012) Patient with cervical adenocarcinoma requires aggressive therapy protocols with combining chemotherapy and radiotherapy even possible with targeted therapy. (Noordhuis et al., 2011; Tang et al., 2012; Barbu et al., 2013) .The results of this study were expected

to obtain update for developing target therapies for patients with cervical adenocarcinoma. In this study, we conduct investigation of the immunohistochemical expression of EGFR and their correlation to radiosensitivity in cervical adenocarcinoma.

The expression of EGFR have been related with aggressive behavior, rotten prognosis and decreasing sensitivity of radiotherapy for several types of malignancies, including cervical cancer. (Wright et al., 2013; Barbu et al., 2013) The knotting between epidermal growth factor and its receptor rouses autophosphorylation of EGFR which is provoke two downstream signaling pathways, the RAS-RAF-MAPK-ERK and PI3K/AKT pathway. Those are implicated in legal action corresponded with tumor outgrowth and progression. (Gadducci et al., 2013) Some authors revealed that EGFR expression is correlated to a decrease radiosensitivity, decrease free survival and worned out entirety survival. (Noordhuis et al., 2011; Barbu et al., 2013; Shimada et al., 2013) Phosphorylated EGFR has been adduced to have a role in legal actions beared with carcinogenesis and tumor progression and also in yielding decreasing radiation result. (Noordhuis et al., 2011)

This study shows EGFR expression correlated with response to radiotherapy. EGFR expression is increased in patients with a poor response to radiotherapy. From statistic correlation analysis gained correlation value as 0,800. According to Guilford criteria, the correlation value between two variables have a strong correlation.

Recently, combination EGFR targeted therapy with standard radiotherapy has been tackled as a innovative therapeutic strategy in divers malignacy. (Noordhuis et al., 2011; Barbu et al., 2013; Shimada et al., 2013; Shang et al., 2012). Clinical trial use the monoclonal anti-EGFR inhibitor cetuximab in combination with (chemo)-radiotherapy in cervical cancer are being implemented. (Noordhuis et al., 2011)

In conclusion, our study revealed, expression of EGFR has been correlated with response to radiotherapy in cervical adenocarcinoma patient. Expression of EGFR can be used as a predictive cell biological marker in cervical adenocarcinoma patient. Further study of this marker and their targeting by targeted drugs in combination with chemoradiotherapy or radiotherapy in clinical practise will expectly work up survival rates in advanced stage of cervical adenocarcinoma patients.

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