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Prognostic significance and therapeutic implications of PTEN, EGFR and MAPK/ERK in cervical squamous cell carcinoma

Hanan Lotfy Mohammed[1], Hanaa A Atwa[1], Abdelmonem Awad Hegazy[2], Amira Elwan[3], Nashwa Nawar[3], Mostafa Abdo Salem[4], Ismail I. Khalil[4]

[1]Department of Pathology, Zagazig University, Zagazig, Egypt

[2]Department of Anatomy and Embryology, Zagazig University, Zagazig, Egypt

[3]Department of Medical Oncology, Zagazig University, Zagazig, Egypt

[4]Department of Gynecology and Obstetrics, Zagazig University, Zagazig, Egypt

ABSTRACT

Introduction: There is a considerable need for cervical cancer treatments. The epidermal growth factor receptor (EGFR) and its pathway molecules affect cervical squamous cell carcinoma (CSCC) regarding its prognosis and anti-cancer therapy.

Aims of Study: To investigate the biologic expression of EGFR and the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway mediators in CSCCs, by investigating immunohistochemical expressions of EGFR, extracellular signal–regulated kinases (ERK1/2) and phosphatase and tensin homolog (PTEN), in a trial to assess their prognostic and survival outcome.

Patients and Methods: Immunohistochemical analysis was performed for 35 cervical squamous cell carcinoma specimens to detect three target antigens EGFR, PTEN, and MAPK/ERK1/2.

Results: EGFR and MAPK/ERK1/2 staining showed a significant association with each of the following clinical parameters – increased patient age, high tumor stage, grade and positive lymph nodes (LN). They are also associated with relapse and poor patient survival. PTEN showed a significant association with decreased age, small tumor size, low grade, low stage and negative LN, meaning a good prognostic clinical outcome with less relapse.

Conclusion: Cervical cancer patients with PTEN expression had good prognosis in contrary to those with EGFR and ERK expressions that had poor prognosis. Treatment directions of CSCC might be of great benefits in the form of an improved clinical outcome with EGFR pathway targeted therapy.

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Cervix; carcinoma; prognosis; therapy; immunohistochemistry

Introduction

Cervical cancer is one of the most frequently diagnosed malignancies representing the fourth leading cause of cancer-related death in females' worldwide [1]. The highest mortality rates are found in African and South Asian countries [2]. Cancer cervix associated mortality rates have dramatically declined in developed countries owing to introduction of national cervical cytology screening programs [3]. The standard treatment for locally advanced cervical cancer consists of concurrent chemo radiation. This leads to a five-year survival rate of only 66% [4]. Therefore, there is a considerable need for cervical cancer treatments. The epidermal growth factor receptor (EGFR) is a well characterized target for anti-cancer therapies in many tumors [5]. Its stimulation activates a tyrosine kinase domain that affects multiple cellular functions including growth and replication [6]. EGFR stimulation activates the mitogen-activated protein kinase/extracellu-

Contact Abdelmonem Awad Hegazy ✉ dr.abdelmonemhegazy@yahoo.com Department of Anatomy and Embryology, Zagazig University, Zagazig, Egypt.

lar signal-regulated kinase (MAPK/ERK) signaling cascade. Phosphorylated extracellular signal–regulated kinases (ERK1/2) activates AP-1 family transcription factors such as jun and fos resulting in cyclins expression and cell proliferation [7,8]. EGFR stimulation also activates the phosphatidylinositol 3-kinase (PI3K) involved in a number of cellular functions including cell proliferation, survival, regulation of tumor growth, and angiogenesis. Activation of PI3K occurs via growth factors including EGFR, platelet-derived growth factor receptor, and others. Further evidence showed that Akt (protein kinase B, PKB) is one of the major targets of PI3K [9]. Upon activation, Akt phosphorylates a number of downstream targets for inhibition of pro-apoptotic proteins and blocking apoptosis. PI3K mediates G1 progression, cyclin expression and protein synthesis through activation of Akt/mammalian Target of Rapamycin (mTOR(signaling pathway [10]. Such pathway could be inactivated by phosphatase and tensin homolog (PTEN) [9,10].

PTEN is a well-known tumor suppressor gene that negatively affects PI3K/mTOR signaling leading to cellular death and regulation of cellular growth and replication. Inhibition of PI3K is suggested to suppress tumor growth and sensitize tumor cells to the treatment of chemotherapeutic drugs and radiation as PI3K/PTEN/Akt signaling pathway exerts a key role in tumor genesis. Loss of PTEN expression causes cellular over growth and is associated with many tumors [11].

High expression of EGFR might be involved in poor response to radiotherapy and poor disease free survival (DFS) in patients treated with chemo radiation or surgery. EGFR pathway could be inhibited pharmacologically through two ways: anti-EGFR monoclonal antibodies or specific inhibitors of the EGFR tyrosine kinase domain. Clinical significance of anti-EGFR therapies were detected in lung, colon, and other tumors [5].

This study aimed to examine the immunohistochemical expressions of three target antigens: EGFR, PTEN and MAPK/ERK1/2 in cervical squamous cell carcinoma (CSCC); and to correlate the expressions of these markers with clinicopathological factors and survival of studied cases.

Patients and Methods

Tissues' specimens and preparations

Thirty-five cervical biopsy specimens diagnosed as squamous cell carcinoma were used in the study. The cases were obtained retrospectively from the archives of the Department of Pathology during the period between 2012 and 2014 with follow-up till 2017 at Zagazig University hospitals. Sections (4–5 μm) from the blocks were

Table 1. Clinicopathological features, immunohistochemical markers and outcome of 35 patients with cervical carcinoma.

	All patients (N = 35)	
Characteristics	**No.**	**%**
Age (years)		
Mean ± SD	52.17	±7.15
Median (Range)	54	(40–65)
≤50 years	16	45.7%
>50 years	19	54.3%
Size (cm)		
Mean ± SD	7.98	±2.89
Median (Range)	9	(1–12)
≤4 cm	6	17.1%
>4 cm	29	82.9%
Grade		
Grade I	4	11.4%
Grade II	13	37.1%
Grade III	18	51.4%
LN		
Node negative	19	54.3%
Node positive	16	45.7%
FIGO stage		
Stage IB1	5	14.3%
Stage IIA1	1	2.9%
Stage IIA2	6	17.1%
Stage IIB	7	20%
Stage IIIA	8	22.9%
Stage IIIB	8	22.9%
PTEN		
Mean ± SD	21.42	±33.61
Median (Range)	0	(0–90)
Negative	24	68.6%
Positive	11	31.4%
ERK1/2		
Mean ± SD	50.42	±36.16
Median (Range)	65	(0–90)
Negative	11	31.4%
Positive	24	68.6%
EGFR		
Mean ± SD	46.57	±35.57
Median (Range)	65	(0–90)
Negative	12	34.3%
Positive	23	65.7%
Follow-up duration (months)		
Mean ± SD	27.51	± 9.56
Median (Range)	30	(8–36)
Relapse		
Absent	10	28.6%
Present	25	71.4%
Death		
Alive	14	40%
Died	21	60%

Categorical variables were expressed as number (percentage). Continuous variables were expressed as mean ± SD & median (range).

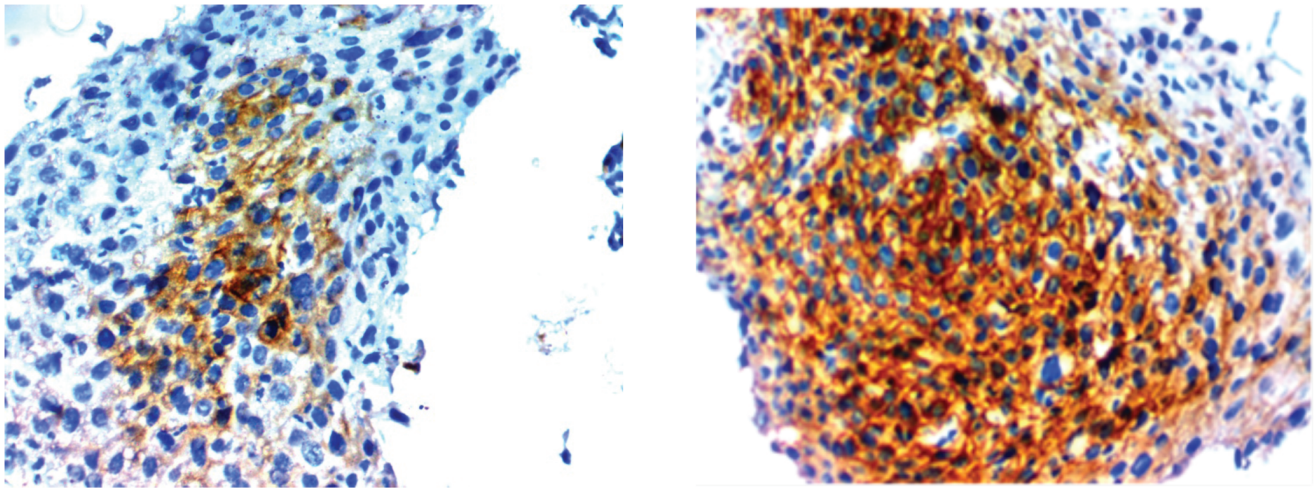


Figure 1. Immunohistochemical stain of EGFR; (a) in low grade CSCC showing mild cytoplasmic and membranous staining; (b) in high grade CSCC showing marked cytoplasmic and membranous staining (× 400).

cut onto slides, air dried overnight, de-paraffinized in xylene, hydrated through a series of graded alcohol and washed in distilled water and 0.01 PBS. The avidin–biotin complex method was used for the immunohistochemistry staining. Primary antibodies were used (to rabbit monoclonal antibody to EGFR, ready to use, SP84, catalog #414R-18, Cell Marque, Rocklin, CA, USA and anti-Pten, rabitt monoclonal antibody 138G6 cell signaling.1\200, anti rabbit monoclonal ab (ERK1/2) #9102 cell signaling 1\100, Danvers, MA, USA). They were reacted with biotinylated anti-mouse antibody (secondary antibody).

Evaluation of immunostaining

For EGFR and ERK1/2 markers, scoring was done as follows: negative, weak (less than 10%), moderate (11 to 50%), and strong (more than 50%) tumor cells stained positive. Immunohistochemical analysis staining intensity was classified mild, moderate, and marked using the following scale: no staining of any cells (0); mild faint staining; moderate intensity staining; and intense staining. EGFR appears as brown membranous and or cytoplasmic staining while ERK1/2 appears as nuclear brown staining [12,13]. Brown cytoplasmic staining less than 30% was negative and more than 30% of the neoplastic cells were considered positive for PTEN [14].

Statistical analysis

Continuous variables were expressed as the mean (±SD) and range (median); and the categorical variables were expressed as a number (percentage). Percent of categorical variables were compared using Pearson's Chi-square test or Fisher's exact test. Overall Survival (OS) was calculated as the time from diagnosis to death or the most recent follow-up contact (censored). The DFS was calculated as the time from start of treatment to date of relapse or the most recent follow-up contact that the patient was known as relapse free. Stratification of OS and DFS was done according which markers were estimated using the method of Kaplan–Meier plot, and compared using two-sided exact log-rank test. A p-value <0.05 was considered significant. All statistics were performed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) and MedCalc windows (MedCalc Software bvba 13, Ostend, Belgium).

Results

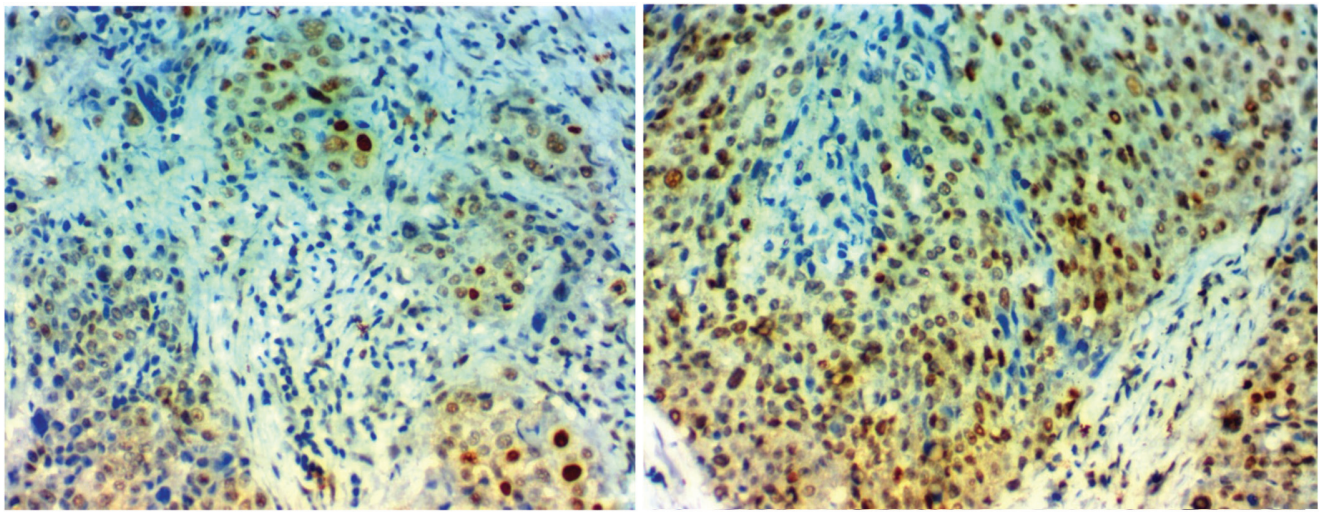
Patients and tumor data

This study included 35 cervical squamous cell carcinoma cases. Patients' age ranged from 40 to 65 years (median 54). The most prevalent grade in the studied cases was grade III CSCC which represented 51.4%. At the same time, grade I represented 11.4% while grade II was 37.1%. Among the 35 cases of CSCCand according to International Federation of Gynecology and Obstetrics (FIGO) staging system, therewere eight cases (22.9%) of stage IIIB, eight cases (22.9%) of stage IIIA, seven cases (20%) of stage IIB, six cases (17%) were IIA2, one case (2.9%) stage IIA1 and five cases (14.3%) were stage of IB1. Positive lymph nodes (LN) were detected in 16 cases (45.7%), while 54.3% of cases had no LN metastasis (Table 1).

Table 2. Relation between clinicopathological features and immunohistochemical staining for PTEN, ERK1/2, and EGFR in 35 patients with cervical carcinoma.

Characteristics	All		PTEN				p-value	ERK1/2				p-value	EGFR				p-value
	(N = 35)		Negative (N = 24)		Positive (N = 11)			Negative (N = 11)		Positive (N = 24)			Negative (N = 12)		Positive (N = 23)		
	No.	(%)	No.	(%)	No.	(%)		No.	(%)	No.	(%)		No.	(%)	No.	(%)	
Age (years)																	
Mean ± SD	52.17	±7.15	53.37	±6.97	49.54	±7.14	0.144*	49.54	±7.14	53.37	±6.97	0.144*	49.33	±6.85	53.65	±6.99	0.090*
Median (Range)	54	(40–65)	54	(43–65)	48	(40–60)		48	(40–60)	54	(43–65)		47.50	(40–60)	54	(43–65)	
≤50 years	16	(45.7%)	9	(56.3%)	7	(43.8%)	0.150‡	7	(43.8%)	9	(56.3%)	0.150‡	8	(50%)	8	(50%)	0.072‡
>50 years	19	(54.3%)	15	(78.9%)	4	(21.1%)		4	(21.1%)	15	(78.9%)		4	(21.1%)	15	(78.9%)	
Size (cm)																	
Mean ± SD	7.98	±2.89	9.60	±1.23	4.45	±2.22	<0.001*	4.45	±2.22	9.60	±1.23	<0.001*	4.75	±2.35	9.67	±1.21	<0.001*
Median (Range)	9	(1–12)	9.75	(7.50–12)	4	(1–7)		4	(1–7)	9.75	(7.50–12)		5	(1–8)	10	(7.50–12)	
≤4 cm	6	(17.1%)	0	(0%)	6	(100%)	<0.001‡	6	(100%)	0	(0%)	<0.001‡	6	(100%)	0	(0%)	0.001‡
>4 cm	29	(82.9%)	24	(82.8%)	5	(17.2%)		5	(17.2%)	24	(82.8%)		6	(20.7%)	23	(79.3%)	
Grade																	
Grade I	4	(11.4%)	0	(0%)	4	(100%)	0.005§	4	(100%)	0	(0%)	0.005§	4	(100%)	0	(0%)	0.003§
Grade II	13	(37.1%)	9	(69.2%)	4	(30.8%)		4	(30.8%)	9	(69.2%)		5	(38.5%)	8	(61.5%)	
Grade III	18	(51.4%)	15	(83.3%)	3	(16.7%)		3	(16.7%)	15	(83.3%)		3	(16.7%)	15	(83.3%)	
LN																	
Node negative	19	(54.3%)	8	(42.1%)	11	(57.9%)	<0.001‡	11	(57.9%)	8	(42.1%)	<0.001‡	12	(63.2%)	7	(36.8%)	0.001‡
Node positive	16	(45.7%)	16	(100%)	0	(0%)		0	(0%)	16	(100%)		0	(0%)	16	(100%)	
FIGO stage																	
Stage IB1	5	(14.3%)	0	(0%)	5	(100%)	<0.001§	5	(100%)	0	(0%)	<0.001§	5	(100%)	0	(0%)	<0.001§
Stage IIA1	1	(2.9%)	0	(0%)	1	(100%)		1	(100%)	0	(0%)		1	(100%)	0	(0%)	
Stage IIA2	6	(17.1%)	1	(16.7%)	5	(83.3%)		5	(83.3%)	1	(16.7%)		5	(83.3%)	1	(16.7%)	
Stage IIB	7	(20%)	7	(100%)	0	(0%)		0	(0%)	7	(100%)		1	(14.3%)	6	(85.7%)	
Stage IIIA	8	(22.9%)	8	(100%)	0	(0%)		0	(0%)	8	(100%)		0	(0%)	8	(100%)	
Stage IIIB	8	(22.9%)	8	(100%)	0	(0%)		0	(0%)	8	(100%)		0	(0%)	8	(100%)	
PTEN																	
Mean ± SD	21.42	±33.61						68.18	±18.34	0	±0	<0.001•	62.50	±26.32	0	±0	<0.001•
Median (Range)	0	(0–90)						55	(50–90)	0	(0–0)		55	(0–90)	0	(0–0)	
Negative	24	(68.6%)						0	(0%)	24	(100%)	<0.001‡	1	(4.2%)	23	(95.8%)	<0.001‡
Positive	11	(31.4%)						11	(100%)	0	(0%)		11	(100%)	0	(0%)	
ERK1/2																	
Mean ± SD	50.42	±36.16	73.54	±12.63	0	±0	<0.001•						3.33	±11.54	75	±10.66	<0.001•
Median (Range)	65	(0–90)	75	(40–90)	0	(0–0)							0	(0–40)	75	(50–90)	
Negative	11	(31.4%)	0	(0%)	11	(100%)	<0.001‡						11	(100%)	0	(0%)	<0.001‡
Positive	24	(68.6%)	24	(100%)	0	(0%)							1	(4.2%)	23	(95.8%)	
EGFR																	
Mean ± SD	46.57	±35.57	67.91	±18.93	0	±0	<0.001•	0	±0	67.91	±18.93	<0.001•					
Median (Range)	65	(0–90)	75	(0–90)	0	(0–0)		0	(0–0)	75	(0–90)						
Negative	12	(34.3%)	1	(8.3%)	11	(91.7%)	<0.001‡	11	(91.7%)	1	(8.3%)	<0.001‡					
Positive	23	(65.7%)	23	(100%)	0	(0%)		0	(0%)	23	(100%)						

Categorical variables were expressed as number (percentage), continuous variables were expressed as mean ± SD and median (range); *Independent samples Student's *t* test; •Mann Whitney *U* test; ‡ Chi-square test; § Chi-square test for trend; $p < 0.05$ is significant.



(a) (b)

Figure 2. Immunohistochemical stains of ERK1/2: (a) in low grade CSCC showing mild nuclear staining; (b) in high grade CSCC showing marked nuclear staining (× 400).

Immunohistochemical results

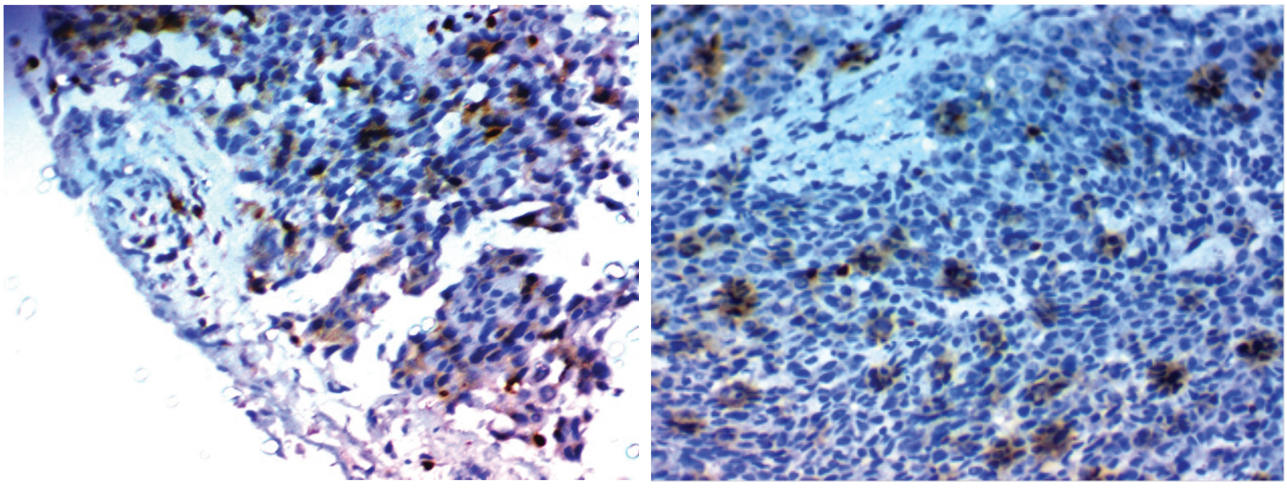
Positive EGFR expression was detected in 23 (65.7%) out of 35 cases of CSCC; and was correlated with increased tumor size ($p < 0.001$), higher grade (p 0.003), high incidence of LN metastases and advanced tumor stage (p <0.001) (Tables 1 and 2; Fig. 1). Positive expression of ERK1/2 was detected in 24 (68.6%) out of 35 cases and was significantly associated with increased tumor size ($p < 0.001$) and higher grade (p 0.005), high incidence of L.N metastases and advanced tumor stage (p <0.001) (Tables 1 and 2; Fig. 2).

Positive PTEN expression was detected in 11 (31.4%) out of 35 cases and unlike the previous markers was correlated with decreased tumor size ($p < 0.001$), lower grade (p 0.005), lower incidence of LN metastases ($p < 0.001$), and early FIGO stage ($p < 0.001$) (Tables 1 and 2; Fig. 3).

Positive EGFR expression showed a significant direct association with ERK1/2 expression (p = <0.001). While the EGFR and ERK1/2 were directly related to each other, both markers were inversely associated to PTEN expression ($p < 0.001$) (Table 2).

Recurrence and survival analysis

Twenty-three out of 35 patients with positive EGFR and ERK showed a significant relapse while only two patients who expressed positive PTEN



(a) (b)

Figure 3. Immunohistochemical stain of PTEN: (a) in low grade CSCC showing moderate cytoplasmic staining; (b) in moderate grade CSCC shows mild cytoplasmic staining (× 400).

Table 3. Relation between clinicopathological features, immunohistochemical staining and outcome in 35 patients with cervical carcinoma.

Characteristics	All		Relapse					Mortality				
	(*N* = 35)		Absent (*N* = 10)		Present (*N* = 25)		*p*-value	Alive (*N* = 14)		Died (*N* = 21)		*p*-value
	No.	(%)	No.	(%)	No.	(%)		No.	(%)	No.	(%)	
Age (years)												
Mean ± SD	52.17	±7.15	4.70	±6.49	53.56	±7.04	0.069*	48.71	±5.41	54.47	±7.34	0.017*
Median (Range)	54	(40–65)	47.50	(40–60)	54	(43–65)		47.50	(40–55)	55	(43–65)	
≤50 years	16	(45.7%)	7	(43.8%)	9	(56.3%)	0.132‡	9	(56.3%)	7	(43.8%)	0.072‡
>50 years	19	(54.3%)	3	(15.8%)	16	(84.2%)		5	(26.3%)	14	(73.7%)	
Size (cm)												
Mean ± SD	7.98	±2.89	4.45	±2.48	9.40	±1.50	<0.001*	5.75	±2.97	9.47	±1.62	<0.001*
Median (Range)	9	(1–12)	3.75	(1–8)	9.50	(6–12)		6.75	(1–10)	10	(6–12)	
≤4 cm	6	(17.1%)	6	(100%)	0	(0%)	<0.001‡	6	(100%)	0	(0%)	0.002‡
>4 cm	29	(82.9%)	4	(13.8%)	25	(86.2%)		8	(27.6%)	21	(72.4%)	
Grade												
Grade I	4	(11.4%)	4	(100%)	0	(0%)		4	(100%)	0	(0%)	
Grade II	13	(37.1%)	5	(38.5%)	8	(61.5%)	<0.001§	7	(53.8%)	6	(46.2%)	0.001§
Grade III	18	(51.4%)	1	(5.6%)	17	(94.4%)		3	(16.7%)	15	(83.3%)	
LN												
Node negative	19	(54.3%)	10	(52.6%)	9	(47.4%)	0.001‡	12	(63.2%)	7	(36.8%)	0.002‡
Node positive	16	(45.7%)	0	(0%)	16	(100%)		2	(12.5%)	14	(87.5%)	
FIGO stage												
Stage IB1	5	(14.3%)	5	(100%)	0	(0%)		5	(100%)	0	(0%)	
Stage IIA1	1	(2.9%)	1	(100%)	0	(0%)		1	(100%)	0	(0%)	
Stage IIA2	6	(17.1%)	3	(50%)	3	(50%)	<0.001§	2	(33.3%)	4	(66.7%)	<0.001§
Stage IIB	7	(20%)	1	(14.3%)	6	(85.7%)		4	(57.1%)	3	(42.9%)	
Stage IIIA	8	(22.9%)	0	(0%)	8	(100%)		2	(25%)	6	(75%)	
Stage IIIB	8	(22.9%)	0	(0%)	8	(100%)		0	(0%)	8	(100%)	
PTEN												
Mean ± SD	21.42	±33.61	64.50	±28.62	4.20	±14.55	<0.001•	42.50	±40.37	7.38	±18.54	0.003•
Median (Range)	0	(0–90)	67.50	(0–90)	0	(0–55)		52.50	(0–90)	0	(0–55)	
Negative	24	(68.6%)	1	(4.2%)	23	(95.8%)	<0.001	6	(25%)	18	(75%)	0.011
Positive	11	(31.4%)	9	(81.8%)	2	(18.2%)		8	(72.7%)	3	(27.3%)	
ERK1/2												
Mean ± SD	50.42	±36.16	4.00	±12.64	69.00	±23.13	<0.001•	26.42	±32.78	66.42	±29.20	0.001•
Median (Range)	65	(0–90)	0	(0–40)	75	(0–90)		0	(0–75)	75	(0–90)	
Negative	11	(31.4%)	9	(81.8%)	2	(18.2%)	<0.001‡	8	(72.7%)	3	(27.3%)	0.011‡
Positive	24	(68.6%)	1	(4.2%)	23	(95.8%)		6	(25%)	18	(75%)	
EGFR												
Mean ± SD	46.57	±35.57	0	±0	65.20	±22.98	<0.001•	20.71	±30.24	63.80	±27.87	<0.001•
Median (Range)	65	(0–90)	0	(0–0)	75	(0–90)		0	(0–75)	75	(0–90)	
Negative	12	(34.3%)	10	(83.3%)	2	(16.7%)	<0.001‡	9	(75%)	3	(25%)	0.004‡
Positive	23	(65.7%)	0	(0%)	23	(100%)		5	(21.7%)	18	(78.3%)	
Relapse												
Absent	12	(34.3%)						9	(90%)	1	(10%)	<0.001‡
Present	23	(65.7%)						5	(20%)	20	(80%)	

Categorical variables were expressed as number (percentage), continuous variables were expressed as mean ± SD and median (range).
*Independent samples Student's *t* test; •Mann Whitney *U* test; ‡ Chi-square test; § Chi-square test for trend; $p < 0.05$ is significant.

showed relapse ($p < 0.001$), with the mean of three years DFS 16.26, 17.08, and 34.50 months for EGFR, ERK, and PTEN, respectively, with significance p <0.001. The mean of three years OS was significantly better in positive PTEN expressed patients with 34.18 months (p 0.005) in comparison to EGFR and ERK positive expressed patients with 23.96 months (p 0.001) and 24.46 months (p 0.005) for EGFR and ERK, respectively (Tables 3 and 4; Fig. 4).

Discussion

This study investigated the changes in EGFR pathway elements in CSCC specimens. There was a positive expression of EGFR correlated with higher grade, high incidence of lymph node metastases,

Table 4. Relation between immunohistochemical staining for PTEN, ERK1/2 and EGFR and outcome in 35 patients with cervical carcinoma.

Characteristics	All (*N* = 35)		PTEN				*p*-value	ERK1/2				*p*-value	EGFR				*p*-value
	No.	%	Negative (*N* = 24)		Positive (*N* = 11)			Negative (*N* = 11)		Positive (*N* = 24)			Negative (*N* = 12)		Positive (*N* = 24)		
			No.	%	No.	%		No.	%	No.	%		No.	%	No.	%	
Relapse																	
Absent	10	28.6%	1	4.2%	9	81.8%	<0.001‡	9	81.8%	1	4.2%	<0.001‡	10	83.3%	0	0%	<0.001‡
Present	25	71.4%	23	95.8%	2	18.2%		2	18.2%	23	95.8%		2	16.7%	23	100%	
DFS																	
Mean (months)	22.54 months		17.08 months		34.50 months		<0.001†	34.50 months		17.08 months		<0.001†	36.64 months		16.26 months		<0.001†
(95% CI)	(18.91–26.16)		(13.61–20.56)		(32.59–36.41)			(32.59–36.41)		(13.61–20.56)			(32.89–36.39)		(12.96–19.56)		
1 year RFS	68.6%		54.2%		100%			100%		54.2%			100%		52.2%		
2 year RFS	42.9%		16.7%		100%			100%		16.7%			100%		13%		
3 year RFS	27.6%		4.2%		80%			80%		4.2%			81.8%		0%		
Death																	
Alive	14	40%	6	25%	8	72.7%	0.011‡	8	72.7%	6	25%	0.001‡	9	75%	5	21.7%	0.004‡
Died	21	60%	18	75%	3	27.3%		3	27.3%	18	75%		3	25%	18	78.3%	
OS																	
Mean (months)	27.51 months		24.46 months		34.18 months		0.005†	34.18 months		24.46 months		0.005†	34.33 months		23.96 months		0.001†
(95% CI)	(24.59–30.64)		(20.56-28.36)		(32.27–36.10)			(32.27–36.10)		(20.56–28.36)			(32.55–36.11)		(20.01–27.90)		
1 year OS	85.7%		79.2%		100%			100%		79.2%			100%		78.3%		
2 year OS	68.6%		54.2%		100%			100%		54.2%			100%		52.2%		
3 year OS	40%		25%		72.7%			72.7%		25%			75%		21.7%		

Continuous variables were expressed as mean (95% CI); categorical variables were expressed as number (percentage); 95%CI: 95%Confidence Interval; ‡ Chi-square test; † Log rank test; $p < 0.05$ is significant.

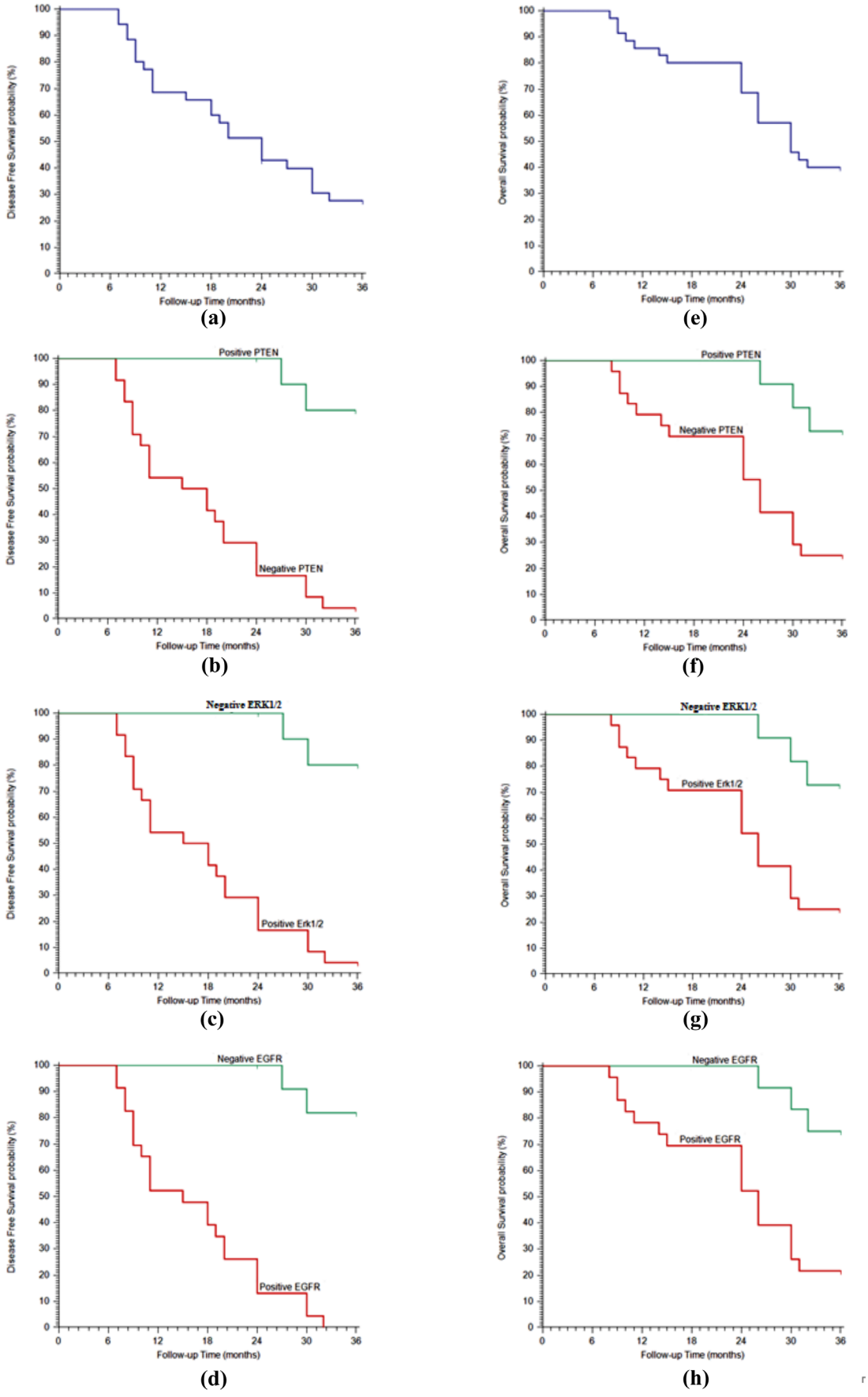


Figure 4. Kaplan Meier plot, Left panel: DFS, Right panel: Overall Survival; (A & E) All studied patients, (B & F) Stratified by PTEN, (C & G) Stratified by ERK1/2 & (D & H) Stratified by EGFR.

and advanced stage of cancer. These findings are close to that of Eijsink et al. [15] who reported that EGFR overexpression was more frequent in advanced cases of CSCC and significantly associated with disease recurrence. The results are also consistent with Li et al. [16] who reported that EGFR over-expression was correlated with increased depth of invasion, LN metastasis, and elevated clinical stage. These findings were explained that EGFR overexpression increased cellular proliferation resulting in uncontrolled and excessive growth and spread of cancer. Tian et al. [17] assessed the correlation between EGFR expression and LN metastasis and tumor size suggesting that high levels of EGFR protein expression might be involved in the progression of cervical cancer which was in agreement with our results. The current study is also compatible with Noordhuis et al. [18] who found a significant association between EGFR expression and high tumor stage and grade which were associated with higher rates of nodal metastasis and decreased patient survival rates.

In this study, positive EGFR expression was found to be associated with poor patient survival, increased disease relapse and death. Our results are close to that of Bumrungthai et al. [13] and Kersemaeker et al. [19] who observed worse prognosis associated with EGFR overexpression. The results are also similar to that of Adimi et al. [12] and Tian et al. [17] who stated that high EGFR expression has shown to be involved in poor response to radiotherapy; and significantly related to poor DFS in patients treated with chemo radiation or surgery. On the contrary to the current study, Kim et al. [20] and Scambia et al. [21] suggested that there is no correlation between EGFR expression and OS and prognosis. Such discrepancy might be due to differences in the immunohistochemical clones and number of examined cases. This necessitates further studies on larger scales.This work revealed a strong association and linear relationship between ERK1/2 and EGFR ($p < 001$). Noordhuis et al. [18] agreed with us; they found a significant correlation between EGFR and ERK staining. This simple linear correlation wasn't detected by Bumrungthai et al. [13]. This contradiction may be due to changes in immunohistochemical protocols.

Results of the present study uncovered that EGFR expression was significantly related to negative PTEN staining which agrees to Noordhuis et al. [18].

The present study disclosed that PTEN expression was significantly associated with, smaller tumor size, lower stage, lower grade and negative LN as detected by Eijsink et al. [15], who disclosed that loss of PTEN expression is associated with pelvic LN metastasis. The absence of expression of this tumor suppressor protein is consistent with tumor progression.The present results are close to Lee et al. [22] who detected that PTEN staining was inversely correlated to grade of differentiation and also with tumor stage. Different results by Loures et al. [23] who found no associations between tumor PTEN expression and tumor stage, grade of differentiation or pelvic lymph node metastasis in CSCC cases, these contradictions may be due to selection of their studied cases which are mostly low grade lesions. Other contradictory results by Bertelsen et al. [14] reported that PTEN is clearly positive in all tumor tissues including the mutated metastatic tumor.

This study showed a significant association between ERK1/2 expression and increased patient age, increased tumor size, tumor grade, FIGO stage, positive LN, positive EGFR staining, and negative PTEN expression. Our results are similar to that of Bai et al. [24] who indicated that ERK1/2 might promote the development of cervical cancer cells. We suggest that EGFR and ERK1/2 inhibitors might be used as an effective target for cervical cancer therapies This is in agreement with Vici et al. [25] who declared that immunohistochemical expression level of molecular pathway targets might be of vital importance for deciding among therapeutic options.

Conclusion

It was concluded that the cervical cancer patients with PTEN expression showed good prognosis in contrary to those with EGFR and ERK expressions showed poor prognosis. Therefore, EGFR pathway targeted therapy could suggest being of great benefits in the treatment directions of squamous cell carcinoma of the cervix with improvement of the clinical outcome.

Further studies on large numbers of patients are recommended to ascertain the benefits of EGFR pathway molecules in treatment of CSCC cases.

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Conflicts of Interest

No commercial or financial conflict of interest.

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