International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Prognostic Benefits of Epithelial Mesenchymal Transition (EMT) markers; Ezrin & Twist-1 Expression in Squamous Cell Carcinoma (SCC) of the Cervix

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Abstract: ***Background: The epithelial mesenchymal transition (EMT) process that has been incriminated in cancer metastases has been extensively studied in several cancers. EMT is controlled by several factors and signaling pathways. Studies about these factors are aiming to detect targeted therapy to several cancer types to improve patients' prognosis. Ezrin&Twist-1 were found to have many roles in EMT and cancer metastases in several cancer types but their role in Squamous Cell Carcinoma (SCC) of the cervix has not been clarified yet. Aim of this study is; to evaluate the prognostic roles of Ezrin & Twist-1 tissue protein expression in Squamous Cell Carcinoma (SCC) of the Cervix. Methods: we have assessed theEzrin & Twist-1 expression using immunohistochemistry in section from 40 paraffin blocks of 40 patients of SCC of the cervix, we have followed the 40 patients for about 3 years aiming to detect the correlations between tissue protein expression of both markers, clincopathological features and patients prognosis. Results: Ezrin expression was positively correlated with increased cancer size, presence of L.N metastases (p=0.003), higher cancer grade (p=0.006), presence of lymphovascular invasion (p=0.004), advanced FIGO stage (p<0.001)and higher incidence of distant metastases (p=0.009).Twist-1 expression was positively correlated with increased cancer size, presence of L.N metastases (p=0.025), higher cancer grade (p=0.004), presence of lymphovascular invasion (p=0.002), advanced FIGO stage (p<0.001)and higher incidence of distant metastases (p=0.006). Cases with increased expression of Ezrin and Twist-1 have higher rate of malignant progression, resistance to chemo-therapy, worse OS& DFS rates and recurrence of the disease after successful therapy (p<0.001). We have found a highly significant correlation between both Ezrin&Twist-1expressions in SCC of the cervix(p<0.001). Table 4; Fig 3. Conclusion; increased tissue protein expression of both Ezrin& Twist-1 is associated with poor clinicopathological and prognostic parameters of patients with SCC of the cervix.***

Keywords: SCC of the cervix,Ezrin, Twist-1, immunohistochemistry, prognosis

1. Introduction

Cancer cervix is the Fourth commonest female cancer worldwide and SCC subtype forms about 90% of all cervical cancers [**Siegel et al., 2014**].

SCC of the cervix is usually managed surgically by radical excision of the tumor with post-operative chemotherapy and/or radiotherapy, but theprognosis of such patients is still dismal especially in advanced and metastatic cases. So, recent studies are trying to discover novel therapeutic modalities aiming at improving the prognosis of patients **[Lin & Chen. 2013**].

The major factor that is responsible for the dismal outcome of such type of cancer is spread and distant metastases which are found to be a multistep process required complicated interactions between cancer and surrounding tissues [**Li et al., 2012**].

The most widely studied mechanism of malignant epithelial cells invasion and metastases is the epithelial-mesenchymal transition (EMT), in which the carcinoma cells might lose their epithelial criteria and they acquire mesenchymal features e.g. motility and spread that allow them to invade the nearby tissue and metastasize distantly **[Bao et al., 2013**]. EMT has been found to be under the control of plethora of factors and signaling pathways that interact together in sequential steps to allow malignant progression. In this study we have evaluated tissue protein expression of two factors that has been incriminated in EMT; Ezrin& Twist-1.

Ezrin is a recently discovered member of ezrin/radixin/moesin (ERM) which found to be the protein that links the cytoskeleton to the cell membrane and it mediates most of the interactions between them e.g. division and motility **[Kong J, et al., 2013**].

Twist-1 is a member of helix-loop-helix family that has an important role in formation of mesoderm and migration of neural crest [**Castanon I and Baylies MK: 2002, Chen ZF and Behringer RR: 1995]**.Roles of Ezrin and Twist-1 in EMT occurrence, invasion and metastases of several cancer types has been studied in many cancers but the detailed role of their tissue expression in SCC of the cervix have not been studied yet.

Aim of this study is; to evaluate the prognostic roles of Ezrin & Twist-1 tissue protein expression in Squamous Cell Carcinoma (SCC) of the Cervix.

2. Patients and Methods

In this study we have assessed the tissue protein marker expression of Ezrin& Twist-1 in 40 patients with SCC of the

cervix using immunohistochemistry. Tissue samples are collected from the patients processed, diagnosed, graded, staged using FIGO system**[Pecorelli S, et al., 2009],** and reviewed by 3pathologists in Pathology department, Faculty of medicine, Zagazig University&Pathology department, Faculty of medicine, Zagazig University. Patients are followed for 3 years in the period from January 2011 to January 2014 for response to therapy, recurrence of the disease and survival rates in Oncology departments.During the period of follow-up we have evaluated Ezrin& Twist-1 tissue protein expression using immunohistochemistry by Streptavidin-biotin method of staining **[Hsu et al., 1981]** we have incubated tissue sections with monoclonal mouse anti-Ezrin (ab4069) antibody ; dilution 1:100, and polyclonal rabbit anti-Twist (ab50581)antibody dilution 1:50 (Abcam, UK, Cambridge). Negative control was done by removal of the primary antibody and replacing it by saline. Slides are assessed and evaluated by 3 pathologists from Pathology department, Faculty of Medicine, Beni-Suef University& Pathology department, Faculty of medicine, Zagazig University.

Evaluation of Ezrin immunohistochemical expression
Ezrin& Twist-1 expressionswere found as brown stain in the cytoplasm and nucleus respectively. Stains extent is the percentage of positive tumor cells in relation to all the sectioned tumor area it is scored into 0= if ≤5% of tumor cells are positive, 1 if 6-25% are positive, 2 if 26-50% are positive and 3 if more than fifty percent of the tumor cells are positive. Stain intensity is the strength of the brown color in the cytoplasm and nuclei and it is scored as 0 if negative, 1 weak, 2 moderate and 3 strong brown stain. To reach a final stain score of both markers expression we multiply values of the intensity of the stain by values of the extent of the stain, which is giving a final stain score of 0-9, we have considered the cutoff value of 3 below which is assessed as low expression and above which is high expression of both markers. **[Li Li et al., 2011, Bajbouj, 2009].**

Statistical analysis
The clinical, pathological, follow up data and markers tissue protein expression values that we have collected were statistically analyzed using SPSS 22.0 for windows and MedCalc-windows.Mann-Whitney-U test for comparison between 2 non-normally distributed variables.

Chi-square test is used for comparison between categorical variables.Kaplan-Meier curve for calculating the Overall-Survival (OS) ratein relation toEzrin&Twist-1 expressions.P-value≤ 0.05 significant, p <0.001 is highly significant while, P> 0.05 is Non-significant.

3. Results

Patient clinicopathological data

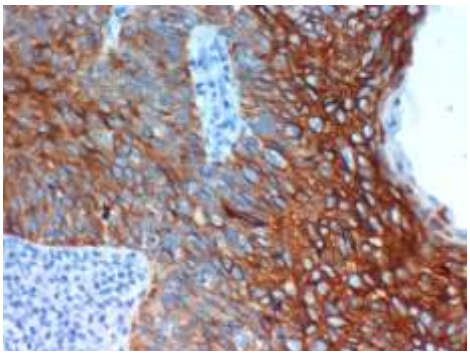
Demographic and follow-up data are included in table 1
In this study we included 40female patients withSCCof the cervix. Median age of our patients is 59 years.

Ezrin expression using immunohistochemistry
Ezrin expression was upregulated in 22 / 40 (55%) patients and it was positively correlated with increased cancer size, presence of L.N metastases(p=0.003), higher cancer grade(p=0.006),presence of lymphovascular invasion (p=0.004), advanced FIGO stage (p<0.001)and higher incidence of distant metastases (p=0.009).but we have not found any significant associations between Ezrin expression and age of the patients**Tables 2& 3; Fig 1**

Twist-1 expression using immunohistochemistry
Twist-1 expression was upregulated in 17 / 40 (42.5%) patients and it was positively correlated with increased cancer size, presence of L.N metastases(p=0.025), higher cancer grade (p=0.004), presence of lymphovascular invasion (p=0.002), advanced FIGO stage (p<0.001)and higher incidence of distant metastases **(p=0.006).** but we have not found any significant associations between Twist-1 expression and age of the patients**Tables 2& 3; Fig 1**

Follow-up data:
After the follow-up period of a median 30 months, 13/40 (33%) of cases have died, three-year overall survival (OS) rate was found to be 64.2% (95% CI; 27.43-32.68 months), three-year disease free survival (DFS) rate was found to be 51.6% (95% CI; (28.02-32.81 months), twenty-five percent of patients [10/40 patients]; experiencedrecurrence of the disease.

Follow-up and survival results in relation to Ezrin& Twist-1expression
Cases with increased expression ofEzrin and Twist-1 have higher rate of malignant progression, resistance to chemo-therapy, worse OS& DFS rates and recurrence of the disease after successful therapy(p<0.001).
We have found a highly significant correlation between both Ezrin&Twist-1expressions in SCC of the cervix(p<0.001). Table 4; Fig 3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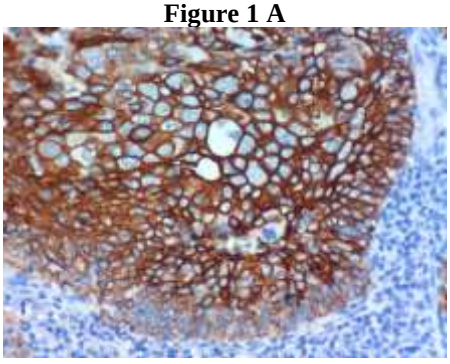
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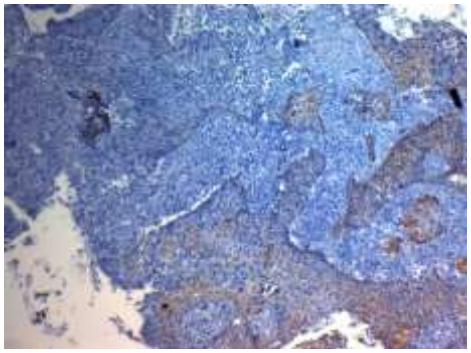


Figure 1 C

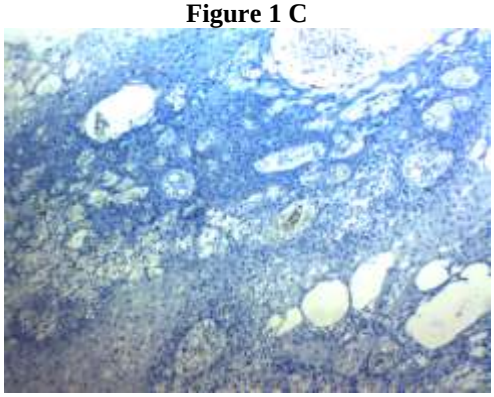
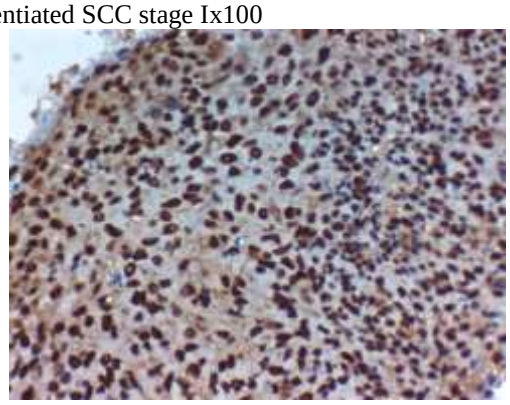


Figure 1 D

Figure1. Immunohistochemical expression of EZRIN in Squamous Cell Carcinoma of the cervix (A) High cytoplasmic expression in poorly differentiated SCC stage IV x400 (B) High cytoplasmic expression in moderately differentiated SCC stage III x400 (C) Low cytoplasmic expression in moderately differentiated SCC stage IIx400 (D) Negative cytoplasmic expression in moderately differentiated SCC stage Ix100



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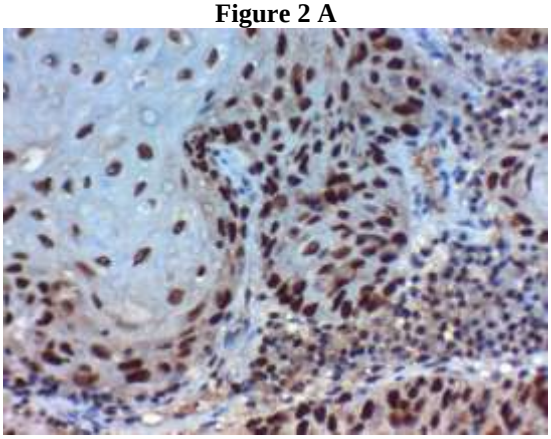

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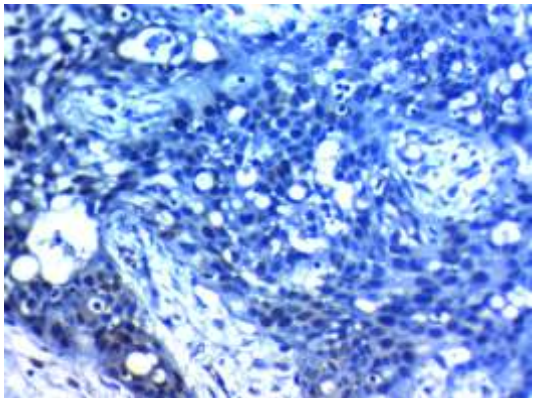

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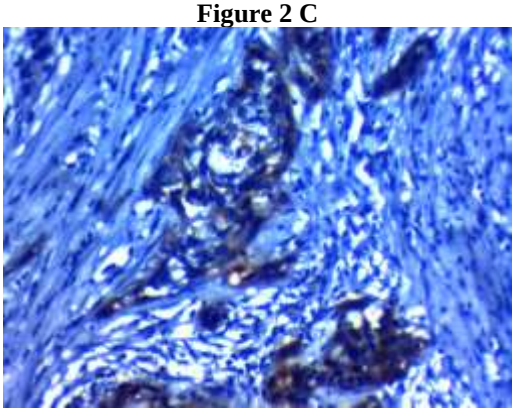


Figure 2 D

Figure 2: Immunohistochemical expression of Twist-1 in Squamous Cell Carcinoma of the cervix (A) High nuclear expression in poorly differentiated SCC stage IVx400 (B) High nuclear expression in moderately differentiated SCC stage III x400 (C) Low nuclear expression in moderately differentiated SCC stage IIx400(D) Low nuclear expression in well differentiated SCC stage Ix400

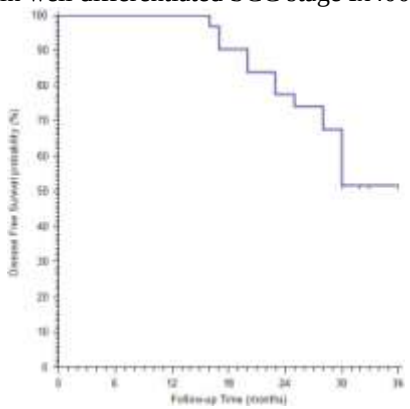


Figure 3 A

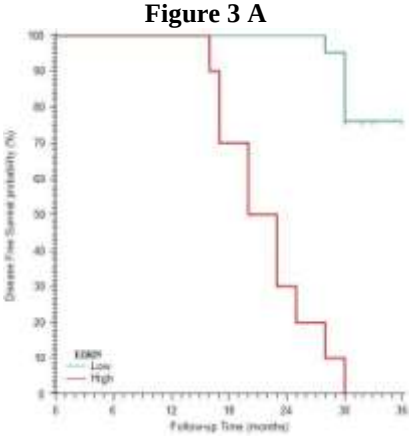
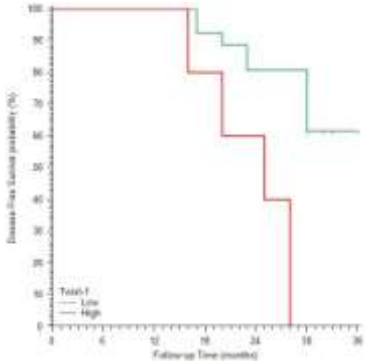


Figure 3 B



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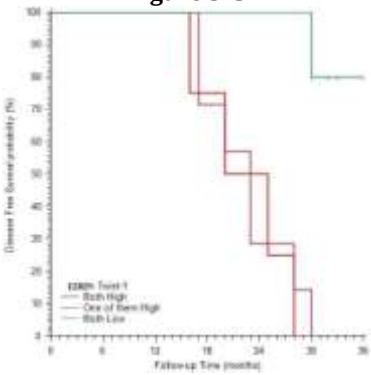


Figure 3 D

Figure (3): Kaplan Meier Survival plots; Disease Free Survival (DFS); (A): All studied patients; (B) Stratified by EZRIN IHC staining; (C) Stratified by Twist-1 IHC staining; (D) Stratified by EZRIN/Twist-1 IHC staining.

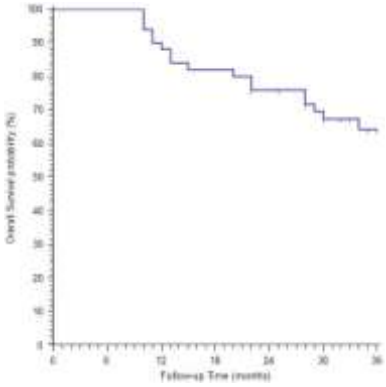
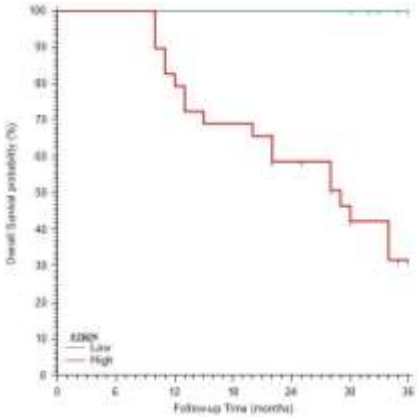


Figure 4 A



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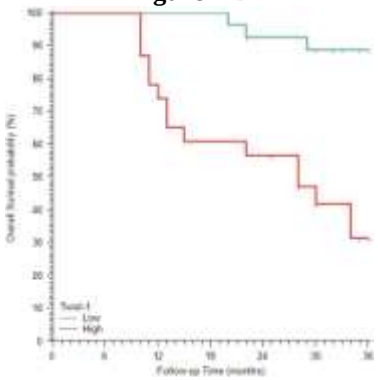

Figure 4 C

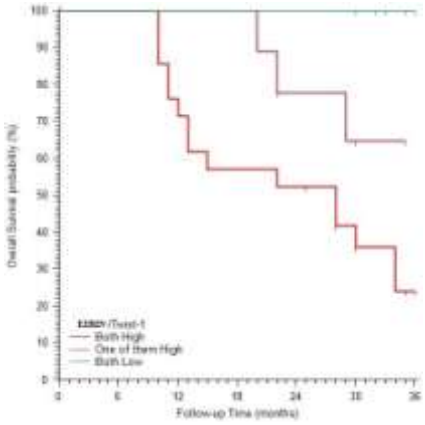

Figure 4 D

Figure (4): Kaplan Meier Survival plots; Overall Survival; (A): All studied patients; (B) Stratified by EZRIN IHC staining; (C) Stratified by Twist-1 IHC staining; (D) Stratified by EZRIN/Twist-1 IHC staining.

Table 1: Clinicopathological features, and outcome of our patients

Characteristics	All (N=40)		Characteristics	All (N=40)	
	No.	(%)		No.	(%)
Age (years)			FIGO stage		
Mean ± SD	59.92	±9.15	Stage I	5	(12.5%)
Median (Range)	59	(40 – 75)	Stage II	17	(42.5%)
<55 years	19	(47.5%)	Stage III	8	(20%)
>55 years	21	(52.5%)	Stage IV	10	(25%)
			Treatment		
			Surgery	5	(12.5%)
			Concurrent CRT	25	(62.5%)
Size			Chemotherapy alone	10	(25%)
<4cm	5	(12.5%)	Response to treatment		
>4cm	35	(87.5%)	CR	20	(50%)
Grade			PR	3	(6%)

Grade I	5	(12.5%)		SD	2	(14%)
Grade II	19	(47.5%)		PD	2	(18%)
Grade III	16	(40%)		N/A	4	(12%)
LVI				OAR	25	(56%)
Absent	30	(75%)		NR	10	(32%)
Present	10	(25%)		N/A	5	(12%)
LN				Follow-up duration (months)		
Negative	25	(62.5%)		Mean ± SD	28.32	±8.97
Positive	15	(37.5%)		Median (Range)	30	(10 – 36)
Distant metastasis				Events		
Negative	30	(75%)		Recurrence	10	(25%)
Positive	10	(25%)		Died	12	(33%)

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

Table 2: Relation between **c**linicopathological features and immunohistochemical markers of our patients

Characteristics	All (N=40)		Ezrin Low (N=18)		High (N=22)		p-value
	No.	(%)	No.	(%)	No.	(%)	
Age (years)							
Mean ± SD	59.92	±9.15	54.80	±8.36	56.72	±8.04	0.418*
Median (Range)	59	(40 – 75)	55	(39-72)	56	(39-72)	
<55 years	19	(47.5%)	10	(47.8%)	9	(52.2%)	0.441‡
>55 years	21	(52.5%)	8	(37%)	13	(63%)	
Size							
<4cm	5	(12.5%)	5	(100%)	0	(0%)	0.003‡
>4cm	35	(87.5%)	13	(34.1%)	22	(65.9%)	
Grade							
Grade I	5	(12.5%)	5	(100%)	0	(0%)	0.006§
Grade II	19	(47.5%)	8	(39.3%)	11	(60.7%)	
Grade III	16	(40%)	5	(22.5%)	11	(100%)	
LVI							
Absent	30	(75%)	18	(56.8%)	12	(43.2%)	0.004‡
Present	10	(25%)	0	(0%)	10	(100%)	
LN							
Negative	25	(62.5%)	15	(74.1%)	10	(25.9%)	0.003‡
Positive	15	(37.5%)	3	(4.3%)	12	(95.7%)	
Distant metastasis							
Negative	30	(75%)	18	(53.8%)	12	(46.2%)	0.009‡
Positive	10	(25%)	0	(0%)	10	(100%)	
FIGO stage							
Stage I	5	(12.5%)	5	(100%)	0	(0%)	<0.001§
Stage II	17	(42.5%)	12	(66.7%)	5	(33.3%)	
Stage III	8	(20%)	1	(8.3%)	7	(91.7%)	
Stage IV	10	(25%)	0	(0%)	10	(100%)	
Twist							
Low	23	(54%)	17	(74.1%)	6	(25.9%)	<0.001‡
High	17	(46%)	1	(4.3%)	16	(95.7%)	

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

Table 3: Relation between **c**linicopathological features and immunohistochemical markers of our patients

Characteristics	All (N=40)		Twist-1 Low (N=23)		High (N=17)		p-value
	No.	(%)	No.	(%)	No.	(%)	
Age (years)							
Mean ± SD	59.92	±9.15	56.55	±8.44	55.17	±7.92	0.566*
Median (Range)	59	(40 – 75)	57	(39-72)	56	(39-70)	
<55 years	19	(47.5%)	12	(52.2%)	7	(47.8%)	0.811‡
>55 years	21	(52.5%)	11	(55.6%)	10	(44.4%)	

Size								
<4cm	5	(12.5%)	5	(100%)	0	(0%)		0.025‡
>4cm	35	(87.5%)	18	(47.7%)	17	(52.3%)		
Grade								
Grade I	5	(12.5%)	5	(100%)	0	(0%)		0.004§
Grade II	19	(47.5%)	10	(53.6%)	9	(46.4%)		
Grade III	16	(40%)	10	(16.7%)	6	(83.3%)		
LVI								
Absent	30	(75%)	20	(70.3%)	10	(29.7%)		0.002‡
Present	10	(25%)	3	(7.7%)	7	(92.3%)		
LN								
Negative	25	(62.5%)	20	(88.9%)	5	(11.1%)		0.025‡
Positive	15	(37.5%)	3	(13%)	12	(87%)		
Distant metastasis								
Negative	30	(75%)	23	(69.2%)	7	(30.8%)		0.006‡
Positive	10	(25%)	0	(0%)	10	(100%)		
FIGO stage								
Stage I	5	(12%)	5	(100%)	0	(0%)		<0.001§
Stage II	17	(42%)	10	(85.7%)	7	(14.3%)		
Stage III	8	(24%)	3	(25%)	5	(75%)		
Stage IV	10	(22%)	0	(0%)	10	(100%)		
Ezrin								
Low	18	(75%)	16	(95.2%)	2	(4.8%)		<0.001‡
High	22	(25%)	7	(24.1%)	15	(75.9%)		

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

Table 4: Relation between immunohistochemical markers and outcome of our patients

Outcome	All (N=40)		Ezrin				p-value	Twist-1				p-value
			Low (N=18)		High (N=22)			Low (N=23)		High (N=17)		
	No.	(%)	No.	(%)	No.	(%)		No.	(%)	No.	(%)	
Treatment												
Surgery	5	(12.5%)	5	(28.6%)	0	(0%)	0.002‡	5	(22.2%)	0	(0%)	0.004‡
Concurrent CRT	25	(62.5%)	10	(71.4%)	15	(69%)		18	(77.8%)	7	(60.9%)	
Chemotherapy alone	10	(25%)	3	(0%)	7	(31%)		0	(0%)	9	(39.1%)	
Response to treatment												
CR	20	(50%)	15	(71.4%)	5	(34.5%)	<0.001‡	12	(74.1%)	8	(21.7%)	<0.001‡
PR	3	(6%)	0	(0%)	3	(10.3%)		0	(0%)	3	(13%)	
SD	2	(14%)	0	(0%)	2	(24.1%)		1	(3.7%)	6	(26.1%)	
PD	2	(18%)	0	(0%)	2	(31%)		0	(0%)	9	(39.1%)	
N/A	4	(12%)	4	(28.6%)	0	(0%)		6	(22.2%)	0	(0%)	
OAR	25	(56%)	13	(71.4%)	12	(44.8%)	<0.001‡	20	(74.1%)	3	(34.8%)	<0.001‡
NR	10	(32%)	0	(0%)	10	(55.2%)		1	(3.7%)	9	(65.2%)	
N/A	5	(12%)	5	(28.6%)	0	(0%)		5	(22.2%)	0	(0%)	
Recurrence												
Absent	18	(42%)	16	(76.2%)	2	(0%)	<0.001‡	18	(59.3%)	0	(0%)	<0.001‡
Present	10	(25%)	2	(23.8%)	8	(34.5%)		10	(37%)	2	(21.7%)	
N/A	12	(33%)	0	(0%)	12	(65.5%)		1	(3.7%)	12	(78.3%)	
DFS												
Mean (month) (95%CI)	30.42 month (28.02-32.81)		34.48 month (30.30-35.65)		21.90 month (18.94-24.86)		<0.001†	31.77 month (29.35-34.19)		23.40 month (18.78-28.02)		<0.001†
12 month DFS (%)	100%		100%		100%			100%		100%		
24 month DFS (%)	77.4%		100%		30%			80.8%		60%		
36 month DFS (%)	51.6%		76.2%		----			61.5%		----		
Mortality												
Absent	28	(67%)	28	(100%)	12	(41.4%)	<0.001‡	24	(88.9%)	9	(39.1%)	<0.001‡
Present	12	(33%)	0	(0%)	17	(58.6%)		3	(11.1%)	14	(60.9%)	
OS												
Mean (month) (95%CI)	30.06 month (27.43-32.68)		36 month		25.54 month (21.76-29.33)		<0.001†	34.62 month (33.07-36.16)		24.59 month (20.11-29.08)		<0.001†
12 month OS (%)	88%		100%		79.3%			100%		73.9%		
24 month OS (%)	76%		100%		58.6%			92.6%		56.5%		
36 month OS (%)	64.2%		100%		31.8%			88.7%		31.4%		

Categorical variables were expressed as number(percentage); ‡ Chi-square test; † Log rank test; p< 0.05 is significant.

4. Discussion

The case fatality rate of SCC of the cervix had been markedly diminished due to advanced modalities in screening, diagnosis and therapy, but the prognosis of advanced and metastatic cases are still dismal which points to the need of discovering recent therapy to improve the prognosis. In our study we have tried to clarify the clinco-pathological and prognostic value of tissue protein Ezrin and Twist-1 expression aiming at using them as therapeutic target for such type of cancer.

In the current study, we assessed the expression of Ezrin in SCC of the cervix and we have proved the association of such marker expression with poor clinicopathological parameters, high incidence of cancer progression, recurrence, poor survival rate and dismal patient outcome.

Our results were nearly similar to results of **Li et al 2017, Kong et al., 2013** who found the same association between Ezrin expression and poor prognosis in cervical cancer patients as its expression was higher in advanced stages and higher grades of cancer cervix.

Kong et al., 2013 did not find a significant correlation between Ezrin expression and patients' survival rates which was slightly different from us such different results may be due to different number of included patients or variable follow-up period.

Ezrin has an essential role in cellular interaction, linking of the cytoskeleton to the cell membrane, preserving normal cell morphology, migration and motility; hence acting as regulator of signaling molecules [**Arpin et al., 2011) , Fehon et al., (2010)].**

Kong et al., 2013 results proved the role of Ezrin in progression and dismal outcome of cervical cancer patients.

Kong et al., 2016 have stated thatEzrin is incriminated in control the occurrence of EMT, malignant promotion and progression. Subsequently down regulation of Ezrin could be a therapeutic target in cancer and its down regulation might suppress progression of cancer cervix, hence improving its prognosis.

Our results are similar to results of previous results about the association between Ezrin expression, cancer progression, and poor prognosis of various types of cancer [**Deng-Xing Lun et al., 2014],** [**Li et al., 2011**], [**Zhai et al., (2010**], [**Elzagheid et al., (2008**].

Yu et al., 2015 found results that are similar to ours that increased the expression of Ezrin is associated with high incidence of invasion, lymph node and distant metastases, that point to the essential role that Ezrin have played in malignant progression and metastases

In this study we have declared that increased Ezrin expression is associated with decreased OS and DFS rates in patients with SCC of the cervix; that was similar to results of YU et al., 2015 in cancer breast patients.

Although our results explained the association between Ezrin expression and progression of SCC of the cervix but the exact role in malignant progression, invasion and metastases is not fully clarified, but Ezrin role may be explained by its regulation of the EMT in cancer [**Yang et al., 2008**]. The most important step in EMT is down regulation of E-cadherin, and it has been found in plethora of cancer and is subsequently leads to malignant invasion and metastases [**J eanes et al., 2008, Foschini et al., 2013, Wang et al., 2013].** It has been previously found that Ezrin knockout could lead to increase the level of E-cadherin expression in plethora of cancers, which might lead to inhibition of EMT, malignant cell invasion and metastases and improve paients prognosis [**Saito et al., 2013**, **Li et al., 2008**].

In the current study, we assessed the expression of another EMT marker in SCC of the cervix that is Twist-1 and we have proved the association of such marker expression with poor clinicopathological parameters, high incidence of cancer progression, recurrence, poor survival rate and dismal patient outcome.

So we have declared that Twist-1 might be considered a useful prognostic and therapeutic target in SCC of the cervix which might improve patients' prognosis. Our results are similar to results of **Qiong Fan et al., 2015 that proved that Twist-1** by regulating the TGF-β/Smad3 signaling pathway could be able to mediate cancer cervix progression invasion and spread. Moreover our results are similar to results of **Shibata et al., 2008** that have demonstrated the association between increased Twist-1 expression in cancer cervix and cancer progression.

Kyo et al. study have clarified that in endometrial carcinoma increased Twist-1 expression have been found to be related to deep invasion, metastases, recurrence and dismal patient outcomel [**Kyo et al., 2006**].

Similar to our results**AlimujiangWushou et al., 2015** meta-analysis who proved the association between Twist-1 expression, cancer progression and poor outcome of patients, additionally Twist-1 might be beneficial as recent predictive and prognostic markers in carcinoma of various types.

Lei Y, *et al*: 2015 explained our results by that increased Twist-1 expression played major roles in EMT by down regulating Ecadherin levels thus increased cancer cells invasion and metastases. Moreover increased Twist-1 expression stimulate the transforming growth factor-β (TGF-β) that found to play many vital roles in EMT induction and cancer progression (**Lamouille S and Derynck R, 2007**). In late stages of carcinoma of the uterine cervix, TGF-β1 extracellular levels were increased (**Comerci et al., 1996].** TGF-β induced EMT is down regulated by inhibition of Twist **Qiong Fan1 et al., 2015**

K. Shibata H et al., 2008 results revealed no association between Twist-1 expression levels and pathological parameters which was different from our results but the found the same association of increased Twist-1 expression and poor patients' survival rates in cancer cervix**.**

Different results of K. Shibata H et al., 2008 may be due to different number of the included cases in their study or different antibody.

Many previous studies have detailed the role of Twist-1 expression in cancer cells invasion and metastases [**Yong et al., 2004, Elias al. 2005, Mironchik et al., 2005**], and proved the association between its expression and poor clinicopathological criteria in cancer patients [**Ken, *et al*. 2009, Soini, *et al*. 2011, Wushou, *et al*., 2012].**

Twist-1 over expression stimulate the occurrence of EMT which subsequently lead to increased malignant cells invasion and metastases, so it is responsible for poor out come in several cancer types, additionally Twist-1 associated with chemoresistance in many cancer types [**Wang et al., 2004],** by inhibiting malignant cells apoptosis by the chemotherapeutic agents **due to induction of of**Akt-dependent anti-apoptotic signaling pathway [**[Zhang et al. 2007]**. Moreover, Twist-1 might affect p53-associated genes expression in breast cancer cells, which subsequently will affect response of such cells to radiotherapy [**Stasinopoulos et al., 2005**]. We found that Twist-1 expression was associated with resistance to chemo-radiotherapy in the group of patients that needed adjuvant therapy post-operatively as a part of their management, so, tissue protein expression levels of Twist-1 affect patient prognosis. Moreover *Twist-1* could be used as a therapeutic target for management of cancer patients [**Zhang, *et al*. 2014**]. Results of our study are similar to results of many studies who declared the association between *Twist-1* expression levels and patient prognosis in plethora of carcinomas types [**Hosono, *et al*. 2014, Kyo, S *et al*. 2006, Hung *et al*. 2009, Lee, *et al*. 2014, Wushou, *et al*..2012**].

Additionally, **Izadpanah MH. et al 2017,** found that Twist-1 up-regulate OCT4 that is a stem cell marker so it was associated with poor prognosis of esophageal squamous cell carcinoma that was similar to our results.

We have correlated the expression levels of Ezrin and Twist-1 in SCC of the cervix with each other and we have found positive correlation between them and found the combined expression of them is associated with cancer progression and dismal outcome in our patients.

5. Conclusions

The tissue protein expression levels of Ezrin &Twist-1 in SCC of the cervix is associated with poor prognosis of the patients so both of them could be used as therapeutic target in such type of cancer.

6. Recommendations

As we are the first study that combines the expression of both Ezrin &Twist-1 in SCC of the cervix we recommend doing another study regarding the prognostic role of both markers in large number of patients with cancer to prove our results.

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