

## Evaluation of Epithelial Mesenchymal Transition Markers E-Cadherin and Vimentin in Carcinoma Cervix

Naveen Kumar R<sup>1</sup>, Charanjeet Ahluwalia<sup>1\*</sup>, Sunita Malik<sup>2</sup> and Rashmi Arora<sup>1</sup>

<sup>1</sup>Department of Pathology, Vardhman Mahavir Medical College and Safdarjung Hospital

<sup>2</sup>Department of Obstetrics and Gynecology, Vardhman Mahavir Medical College and Safdarjung Hospital

### ABSTRACT

**Background:** Cervical cancer is the second most common in developing areas. Epithelial to mesenchymal transformation, one of the critical elements in invasion, progression and metastasis of tumour. This study highlights the expression of epithelial mesenchymal markers E-cadherin and vimentin in carcinoma cervix and whether there is any association of expression of these markers with grade of cervical cancer.

#### Objectives:

1. To study the expression of epithelial mesenchymal transition (EMT) markers E-cadherin and vimentin in cervical cancer.
2. To correlate the immunohistochemical expression of these markers with grade of cervical cancer.

**Methods:** 30 cases (n=30) of carcinoma cervix & 30 controls (n=30) of non specific cervicitis diagnosed on H&E were included in this study. H&E stained sections was examined for histological type and grade. Immunohistochemistry for E-Cadherin and Vimentin was performed in all these cases.

**Result:** Immunohistochemical expression of epithelial marker E-cadherin and of mesenchymal marker vimentin was correlated with the grade of cervical carcinoma. The expression of E-cadherin is reduced and expression of vimentin is increased with increasing grades of carcinoma cervix.

**Conclusion:** The expression of these EMT markers can be used as a prognostic marker in cervical cancer that are in high risk of progression.

**Keywords:** Cervical carcinoma, Epithelial Mesenchymal Transition, Squamous Cell Carcinoma, Immunohistochemistry, E-Cadherin, Vimentin.

### Introduction

Cervical cancer is the fourth most common cancer in women globally and the second most common in developing areas. [1] In India, there are 20.2 per 100000 new cases of Cervical cancer diagnosed per year and 11.1 per 100000 deaths annually, accounting for more than one fifth of the global cervical cancer deaths. [2]

Epidemiological studies have identified a number of risk factors for cervical cancer, such as infection with certain oncogenic types of human papillomaviruses (HPV), sexual intercourse at an early age, multiple sexual partners, multiparity, long-term oral contraceptive use, tobacco smoking, low socio-economic status, infection with Chlamydia trachomatis, micronutrient deficiency, and a diet deficient in vegetables and fruits, that contribute to the development of cervical cancer. [3,4]

Epithelial to mesenchymal transformation, one of the critical elements in invasion, progression and metastasis, is a process by which epithelial tumour cells lose their polarity and are converted to a mesenchymal phenotype. [5,6]

E-cadherin is a calcium-dependent transmembrane glycoprotein which functions as an adhesion molecule. It is present in most epithelial cells in embryonic & adult tissues. The cells undergoing EMT downregulate E-cadherin. The occurrence of an altered E-cadherin expression has been correlated with histological differentiation, increased risk of local invasion and metastatic disease as well as poor prognosis. [7]

Vimentin is a type III intermediate filament protein that is expressed in mesenchymal cells. Vimentin plays a significant role in supporting and anchoring the position of the organelles in the cytosol. It is often used as a marker of mesenchymally derived cells or cells undergoing an epithelial to mesenchymal transition (EMT) during both normal development and metastatic progression where it is upregulated. High expression of vimentin is seen as an indicator of an advanced disease with a poorer prognosis. [8]

The expression of E-cadherin and Vimentin could help to predict the prognosis and these biomolecules can be used as biomarkers for further research on the micro-invasion of the tumour for early diagnosis and survival of the patients.

This study highlights the expression of epithelial mesenchymal markers E-cadherin and vimentin in carcinoma cervix and association of expression of these markers with grade (histological differentiation) of cervical cancer.

## Materials and Methods

30 cases and 30 controls were included in this study. The ethical clearance was obtained from the institution where the study took place. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975 as revised in 2000. This was a cross-sectional study

### Inclusion Criteria

All histopathologically proven cases of carcinoma cervix from biopsy and hysterectomy specimens were included in the study.

### Exclusion Criteria

Patients on chemotherapy or radiotherapy, sarcomas and metastatic carcinoma to cervix were excluded from the study.

The samples were subjected to routine histological processing followed by hematoxylin and eosin staining. H&E stained sections were examined for histological type and grade. All cases were divided into well, moderately and poorly differentiated squamous cell carcinoma and adenocarcinoma. All controls were diagnosed as chronic cervicitis. Sections of 3µm thickness taken on Poly-L-lysine coated slides were prepared for each specimen. Immunohistochemistry analysis was done for both E-Cadherin and Vimentin on all cases and controls.

### Immunoreactivity Score

Immunoreactivity was evaluated semi-quantitatively on the basis of staining intensity and distribution using immunoreactive score: intensity score x proportion score.<sup>[10]</sup>(table 1)

Cells positive for E-Cadherin showed a membranous positivity. Vimentin on the other hand showed a diffuse cytoplasmic positivity. Result of H&E staining and the expression of E-Cadherin & vimentin immunohistochemistry were compared in cervical cancer. IHC expression of these markers were statistically correlated with grade of the tumour.

**Statistical Analysis:** Quantitative variables were compared using Independent T test/Mann-Whitney Test between the two groups and ANOVA/Kruskal Wallis test between three groups. Qualitative variables were correlated using

Chi-Square test/Fisher exact test. A p value of  $\leq 0.05$  was considered statistically significant.

## Result

Out of 30 cases of carcinoma cervix, 27 (90%) cases were diagnosed as squamous cell carcinoma and 3 (10%) were diagnosed as adenocarcinoma. All controls were histopathologically diagnosed as chronic cervicitis (100%).

The overall age of the patients in our study ranged from 35 to 70 years with mean age of patients was  $51.97 \pm 9.43$  years and median age was 51 years. The age range in controls of chronic cervicitis was from 38 to 62 years.

All 30 cases of carcinoma cervix were divided into three grades according to the degree of differentiation of tumours - Well differentiated, Moderately differentiated and Poorly differentiated. Out of 30 cases 8 cases (26.67%) were well differentiated carcinomas, 10 cases (33.33%) were moderately differentiated tumours and 12 cases (40%) were poorly differentiated tumours.

IHC was performed and E-Cadherin expression was studied in all the cases and controls. Out of 30 cases of carcinoma cervix, 18 cases (60%) were positive (figure 1) and 12 cases (40%) were negative for E-Cadherin (figure 2). All the 30 controls (100%) showed expression of E-Cadherin. This correlation was statistically significant with a p-value of  $\leq 0.05$ .

E-Cadherin expression was correlated with histological grade of the carcinoma cervix. All 8 cases (100%) of well differentiated tumours were positive for E-Cadherin. Out of 10 moderately differentiated tumours, 8 (80%) were positive and 2 (20%) were negative for E-Cadherin. Among 12 poorly differentiated tumours, 2 cases (16.67%) were positive and 10 cases (83.33%) were negative for E-Cadherin. Thus, it was observed that the expression of E-cadherin reduced with increase in the grade of the tumour. This association was statistically significant with a p-value of  $< .05$ .

Immunoreactivity score of E-Cadherin expression was evaluated semiquantitatively on the basis of staining intensity and distribution using immunoreactive score: intensity score x proportion score. Of 30 cases of carcinoma cervix, 8 cases (26.67%) showed high immunoreactive score, 10 cases (33.33%) showed low immunoreactivity and 12 cases (40%) were negative. Of the 30 controls of chronic cervicitis, all showed high immunoreactive score.

E-Cadherin immunoreactivity score of were also correlated with histological grades of cervical carcinoma which showed a decrease in immunoreactive score of E-Cadherin immunoreaction with increasing grade of carcinoma cervix

and this association was found to be statistically significant with a p-value  $\leq 0.05$  (table 2).

Similarly, the IHC expression of vimentin was studied in 30 cases of carcinoma cervix and 30 controls of chronic cervicitis. All 30 controls of chronic cervicitis showed no expression of vimentin in epithelial cells. Among 30 cases of carcinoma cervix, 23 cases (76.67%) were positive for vimentin (figure 3) and 7 cases (23.33%) were negative for vimentin (figure 4). This vimentin expression was statistically significant with p-value of  $\leq .05$ .

Vimentin expression was also correlated with histological grade of the carcinoma cervix which showed the

expression of vimentin increased with an increase in the histological grade of the tumour. This association of vimentin expression with histological grade of the tumours was found to be statistically significant with a p-value of  $\leq .05$  (table 3).

The immunoreactivity of Vimentin were also correlated with histological grades of cervical carcinoma which showed increased expression of Vimentin immunoreactivity with increasing grades of carcinoma cervix. This association of vimentin immunoreactivity with histological grade of the tumours was found to be statistically significant with a p-value  $\leq 0.05$  (table 4).

**Table 1: Immunoreactivity score.**

Intensity Score	
0	Negative
1	Weak
2	Moderate
3	Strong
Proportion Score	
0	Negative
1	10% (or) less
2	11% to 50%
3	51% to 80%
4	80% (or) more positive cells
Total Immunoreactive Score	
TOTAL SCORE	IMMUNOREACTIVITY
0	Negative immunoreactivity
1 to 4	Low immunoreactivity
>4	High immunoreactivity
The total score ranges from 0 to 12.	

**Table 2: Association of E-Cadherin immunoreactive score with grade of tumours.**

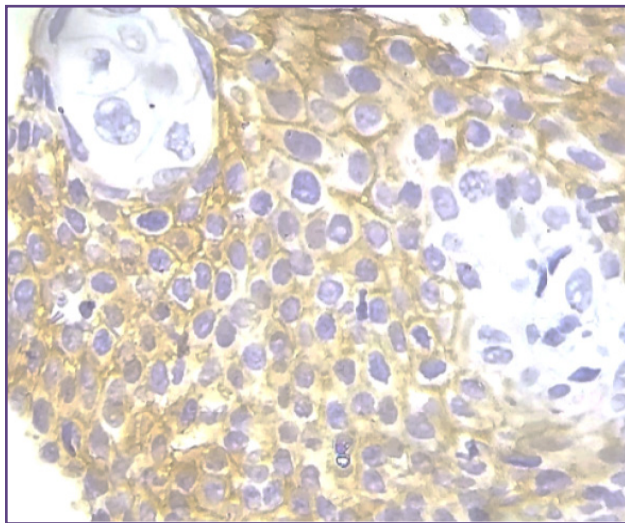
IMMUNOREACTIVITY		GRADE			Total	P value
		PD	MD	WD		
ECADHERIN	HIGH	0 (0.00%)	0 (0.00%)	8 (100.00%)	8(26.67%)	< .05
	LOW	2 (16.67%)	8 (80.00%)	0 (0.00%)	10(33.33%)	
	NEGATIVE	10 (83.33%)	2 (20.00%)	0 (0.00%)	12(40.00%)	
<b>Total</b>		<b>12 (100.00%)</b>	<b>10 (100.00%)</b>	<b>8 (100.00%)</b>	<b>30(100.00%)</b>	

**Table 3: Correlation of vimentin expression with histological grade of carcinoma cervix.**

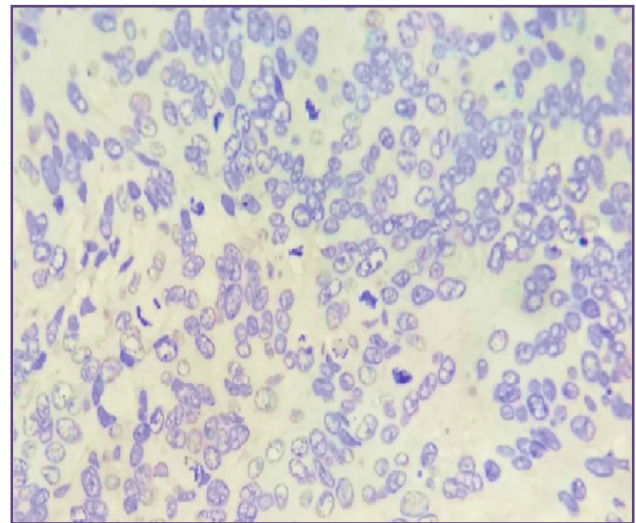
		GRADE			Total	P value
		P D	M D	W D		
VIMENTIN	NEGATIVE	0 (0.00%)	0 (0.00%)	7 (87.50%)	7 (23.33%)	< .05
	POSITIVE	12 (100.00%)	10 (100.00%)	1 (12.50%)	23 (76.67%)	
<b>Total</b>		<b>12 (100.00%)</b>	<b>10 (100.00%)</b>	<b>8 (100.00%)</b>	<b>30 (100.00%)</b>	

**Table 4: Association of vimentin immunoreactivity with histological grade of tumour.**

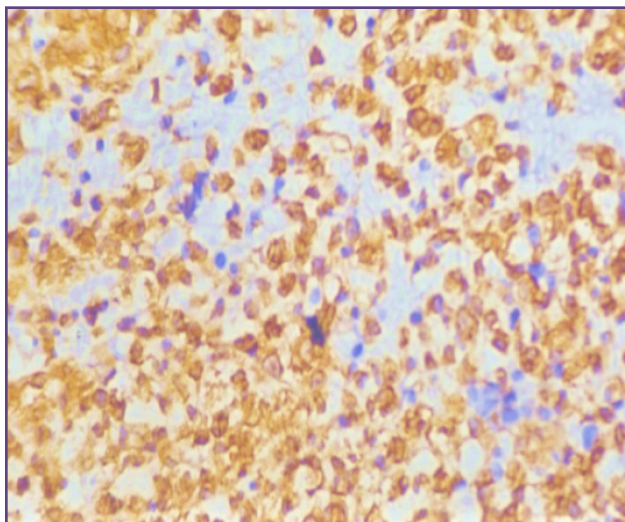
Immunoreactivity		GRADE			Total	P value
		P D	M D	W D		
Vimentin	High	12 (100.00%)	9 (90.00%)	0 (0.00%)	21(70.00%)	< .05
	Low	0 (0.00%)	1 (10.00%)	1 (12.50%)	2 (6.67%)	
	Negative	0 (0.00%)	0 (0.00%)	7 (87.50%)	7 (23.33%)	
<b>Total</b>		<b>12 (100.00%)</b>	<b>10 (100.00%)</b>	<b>8 (100.00%)</b>	<b>30 (100.00%)</b>	



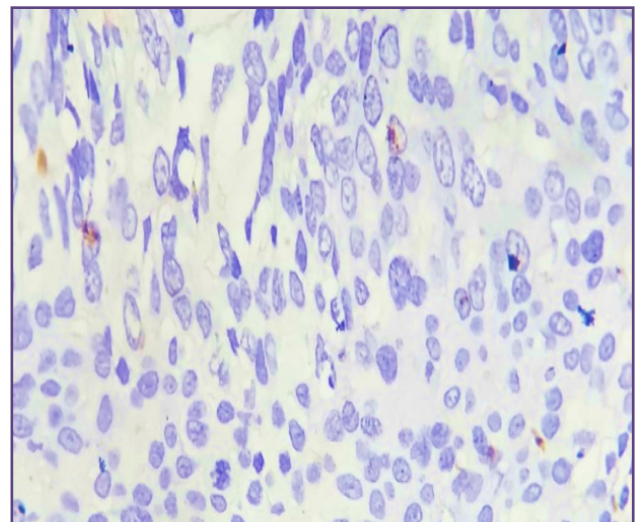
**Fig. 1: E-Cadherin membranous positivity in well differentiated SCC (IHC x 400).**



**Fig. 2: E-Cadherin negative in poorly differentiated SCC (IHC x 400).**



**Fig. 3:Vimentin positive in poorly differentiated SCC (IHC x400).**



**Fig. 4: Vimentin negative in moderately differentiated SCC (IHC x400).**

## Discussion

Epithelial to Mesenchymal transition is a critical process in which the polarized epithelial tumour cells convert into motile mesenchymal cells, invade the basement membrane beneath, enter blood vessels, and disseminate into secondary organ. This epithelial to mesenchymal transition is major cause of mortality in many cancers including cervical cancer. There is downregulation of epithelial markers and upregulation of mesenchymal markers with increasing grades of tumours.<sup>[11]</sup>

In the present study we classified the tumours as squamous cell carcinoma and adenocarcinoma. Out of 30 cases 27 cases (90%) were squamous cell carcinoma and 3 cases (10%) were adenocarcinoma. According to literature, squamous cell carcinoma is most common type of cancer comprising of 80-90% and adenocarcinoma is less common comprising of 10-20%.

In our study E-Cadherin expression was strong in all 30 controls. Out of 30 cases of carcinoma cervix, 18 cases (60%) were positive and 12 cases (40%) were negative for E-Cadherin. We also correlated the expression of E-Cadherin with histological grades of tumours. E-Cadherin was positive in all 8 cases (100%) of well differentiated tumours with a high immunoreactivity. Out of 10 moderately differentiated tumours 8 cases (80%) were positive with low immunoreactivity and 2 cases (20%) were negative. Among 12 poorly differentiated tumours 2 cases (16.67%) were positive with low immunoreactivity and 10 cases (83.33%) were negative. Thus as the grade of the tumour increased, the expression of E-cadherin decreased and this was statistically significant.

Vimentin expression was negative in all 30 chronic cervicitis controls. Out of 30 cases of carcinoma cervix, 23 cases (76.67%) were positive for vimentin and 7 cases (23.33%) showed no expression of vimentin. On correlating vimentin expression with histological grade, we observed that vimentin expression was positive in all 12 cases (100%) of poorly differentiated tumours with high immunoreactivity. All 10 moderately differentiated tumours (100%) were positive for vimentin, of which 9 cases (90%) showed high immunoreactivity and 1 case (10%) showed low immunoreactivity. Among 8 well differentiated tumours 1 case (12.50%) was positive with low immunoreactivity and 7 cases (87.50%) were negative. Thus, the expression of vimentin increased with an increase in the grade of the tumour which was statistically significant with a p-value  $\leq 0.05$ .

Similar study evaluated significance of E-cadherin,  $\beta$ -catenin, and vimentin expression as postoperative

prognosis indicators in cervical squamous cell carcinoma. They examined 135 squamous cell carcinomas cases and 55 normal cervical tissues as controls. High expression of E-cadherin and  $\beta$ -catenin and negative expression of vimentin were found in all of the 55 normal cervical tissues. In cancer tissue, E-cadherin and  $\beta$ -catenin staining was strong in well-differentiated tumours and decreased in moderately differentiated carcinoma, with weak to negative staining in poorly differentiated carcinomas. Thus, the absence or low expression of E-cadherin was observed in 97% of samples from patients who eventually developed a recurrent cancer and 100% of cancers from patients who died of recurrence.

Increased expression of vimentin was observed in 87% of cancers from both patients who eventually developed recurrence and patients who died of recurrence. Similar to our study the expression of vimentin was inversely associated with that of both E-cadherin and  $\beta$ -catenin (P value  $\leq 0.05$ ).<sup>[10]</sup>

Another study evaluated the expression of E-cadherin and Vimentin, and their significance in cervical cancer metastasis. They evaluated 111 premalignant cases and 189 malignant cases and correlated the immunohistochemical expression of E-cadherin and vimentin to predict invasiveness and their prognostic importance. Similar to our study they found that E-cadherin showed a significant progressive loss of staining as the tumour differentiated from a well differentiated grade to a poorly differentiated grade whereas progressive gain of Vimentin was seen with increasing grade of the tumour which were statistically significant.<sup>[9]</sup>

These findings provided significant evidence in favour of the role of epithelial mesenchymal transition (EMT) in progression of cancer cervix. In our study also there was decrease in E-Cadherin expression and increase in vimentin expression as the tumour grade increased from well differentiated to poorly differentiated tumour which was statistically significant. Since these markers form part of the spectrum of changes associated with EMT, the study establishes proof of concept of existence of this process in vivo.

## Conclusion

The expression of these EMT markers can be used as a prognostic marker in cervical cancer that are in high risk of progression. These EMT markers have the ability to identify patients at increased risk of progression and metastasis in cervical cancer. Once identified, the high risk patients could be offered more aggressive treatment options and more intensive follow-up. However larger cohorts are needed to study their biological impact in the course of

the disease of which could lead to improved survival of cervical carcinoma in future.

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None declared

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### \*Corresponding author:

**Dr. Charanjeet Ahluwalia**, 4th floor, Department of Pathology, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi- 110029, India.

**Phone:** +91 9811175639

**Email:** charanjeet.ahluwalia@rediffmail.com

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