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Original Research Article

Expression of e-cadherin and Ki-67 in cervical cancer: a study in a tertiary care hospital, Dhaka, Bangladesh

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ABSTRACT

Background: Cervical cancer, ranking as the second most prevalent cancer and a leading cause of female cancer-related deaths in low to middle-income nations, exhibits a notable connection between tumor proliferation (Ki-67) and invasiveness (e-cadherin). Both factors contribute significantly to cervical cancer's aggressiveness. This study aims to establish the clinical-pathological link between e-cadherin and Ki-67 expression in cervical carcinoma, and assess their potential as diagnostic biomarkers.
Methods: This cross-sectional study spanned July 2017 to June 2019 at Sir Salimullah Medical College and Mitford Hospital Dhaka. It encompassed 60 adult female patients histopathologically diagnosed with cervical cancer. All patients underwent e-cadherin, Ki-67 expression assessments, and histopathological diagnosis. Ethical clearance was granted by SSMC Institutional Ethics Committee.
Results: E-cadherin expression presented as follows: strong intensity in 36.7% cases, weak and homogeneous in 35.0%, weak and heterogeneous in 8.3%, negative staining in 15.1%, and negative expression in 5.0%. Ki-67 mean levels varied significantly among different types of cervical cancer ($p<0.05$). Squamous cell carcinoma was predominant (80.0%), with heightened e-cadherin expression in well-differentiated cases (14 cases). Notably, cases with no e-cadherin expression exhibited Ki-67 mean of 71.0±7.9, while those with strong E-cadherin expression displayed Ki-67 mean of 56.5±8.2 ($p<0.05$). A significant negative correlation ($r=-0.300$; $p=0.022$) emerged between Ki-67 LI (%) and e-cadherin expression.
Conclusions: E-cadherin correlates effectively with clinicopathological features and Ki-67 expression in cervical carcinoma. This underscores its pivotal role in cervical cancer progression.

Keywords: Cervical cancer, Biomarkers, Ki-67, E-cadherin, Hysterectomy, Biopsy

INTRODUCTION

Cancer of the cervix uteri is the 4th most common cancer among women worldwide, and 7th overall. Nowadays along with cancer screening programs, various biotechnologies are available to identify cervical lesions early.[1] It is needed to find out efficacious immunomarkers which can be used to evaluate the primary tumor and refine the subset of patients at the most significant risk for recurrence and optimize intensive disease management. Despite that, new predictive biomarkers are needed to find out patients with a high risk of relapse and to optimize disease management such as primarily with surgical therapy and concurrent chemotherapy, especially in the

case of early cervical cancer.[2] Thus, one major field of research was directed towards tumor proliferation The molecular point of tumor aggressiveness is related to: uncontrolled tumor proliferation activity, that can be assessed by several cellular proteins such as proliferating cell nuclear antigen and Ki-67 antigen; and adhesion, migration, and tumor cell invasiveness, related especially to E-cadherin adhesion molecule.[3] Ki-67, a proliferation marker, is defined as a nuclear antigen (associated with hetero- and 7 euchromatin) expressed during all active phases of the cell cycle (G1, S, G2, M) except G0. It acts as a predictive factor for tumor development It is a non-histone protein acting during the cell cycle other than G0.[2] It is well demonstrated that Ki-67 expression is increased in the upper layers of cervical epithelium being of major significance for the differentiation of non-neoplastic lesions that can mimic cancer.[4] It can be used as an independent prognostic factor for the progression and biological behavior of cervical dysplasia, especially when HPV infection assessment is missing.[5] The E-cadherin is a 120 kd transmembrane glycoprotein.[6] It is an adhesion molecule that mediates calcium-dependent, homophilic cell-cell interactions. E-cadherin means the epithelial cadherin which is required for normal cell-cell adhesion and maintaining tissue integrity and homeostasis. E-cadherin has been identified as a tumor suppressor gene.[7] Aberrant expression of classical cadherin was found to be associated with tumor invasion and a worse prognosis in many carcinomas.[8] It is recognized as having a role in the development of cervical cancer. It also has been seen to be associated with tumor invasion and poor prognosis.[9] In cervical cancer, the expression of E-cadherin at both the mRNA and protein levels is reduced especially in invasive carcinoma.[10] Thus, it means E-cadherin expression is silenced by DNA methylation in cervical cancer cell lines and tumors. Decreased expression of E-cadherin is a useful parameter of cervical malignancy.[11] There is often inter-observers' variability in the diagnosis and assessment of the biological behavior of cervical lesions as it confines to the interpretation of histomorphology. Therefore, a simple laboratory method based on the evaluation of proliferative activity and invasiveness of tumor cells on cervical biopsy material would be of significant clinical value.

The objective of this study revolves around understanding the clinico-pathological relationship between e-cadherin and Ki-67 expression in cervical carcinoma, while also assessing the potential clinical significance of e-cadherin and Ki-67 as diagnostic biomarkers for cervical cancer. The specific aims of this research are as follows: first, to explore the connection between the mean Ki-67 levels in the study participants and their corresponding histopathological diagnoses; second, to analyze the distribution of the study participants based on the degree of e-cadherin expression; third, to investigate the correlation between the degree of e-cadherin expression and the histopathological diagnosis; and fourth, to compare the Ki-67 labeling index and e-cadherin staining pattern across various histopathological diagnoses within cervical cancer.

METHODS

This cross-sectional study was carried out at the department of pathology, Sir Salimullah Medical College, and Mitford Hospital Dhaka. The study was conducted for two years starting from 01 July 2017 to 30 June 2019. A total of 60 cervical biopsies and hysterectomies specimens with histologically confirmed carcinoma of the cervix were selected purposively following the inclusion and exclusion criteria. Adult female patients with a confirmed histopathological diagnosis who provided informed consent were included in the study. On the other hand, patients presenting concurrent pathologies involving organs such as the uterus and ovaries alongside the cervix, tissue blocks displaying extensive necrosis or hemorrhage, patients with a history of prior cervical cancer chemotherapy and radiotherapy, patients diagnosed with metastatic cervical carcinoma, and those who declined participation were excluded from the study. The surgical specimens were fixed in 10% formalin, processed, embedded in paraffin, and stained with hematoxylin–eosin. The sections were stained with mouse monoclonal antibodies against e-cadherin. Then envision dual link system-HRP (ready to use; Dako), be used as a secondary antibody. Incubation with 3, 3-diaminobenzidine tetrahydrochloride. (DAB) will be performed for 10 min as a substrate chromogen solution to produce a brown color. Finally, the sections were counterstained with Mayer's hematoxylin. Ki-67 expression was detected through an immune histochemical stain using a specific monoclonal antibody. A mouse monoclonal antibody against Ki 67 antigen (1:100, MIB-1, Dako, Glostrup, Denmark) was used and the avidin-biotin complex method was employed. By using the monoclonal antibody Ki-67, the cellular proliferation rate was quantitatively investigated. Ki-67 has nuclear staining only. The immune positivity was considered when there was strong brown to black color nuclear staining. More than 50% of positive cells are considered high-grade carcinoma. Ki-67 labeling index (LI) was calculated by the number of cells showing positive staining per 100 cells in separate representative areas of the tumor.

Data analysis was conducted by descriptive and analytical techniques including mean, SD, frequency distribution, computation of percentage. Associations among qualitative and quantitative variables of various factors were studied by using the Chi-square test and t-test where appropriate. Logistic regression analysis, one-way analysis of variance (ANOVA), and correlation were performed to assess the independent relationship between the factors.

A p value <0.05 was considered to be significant. Statistical package for social science (SPSS) version 21 for windows was used to analyze the data. Verbal and written consents were obtained from all participants and ethical clearance was obtained from the Institutional ethical committee of SSMC, Dhaka, Bangladesh.

RESULTS

The age distribution underscored that the 50-59 age bracket emerged as the predominant segment, constituting 48.33% (n=29) of the participants. This was followed by the 60-69 age group, capturing 28.33% (n=17), and the 40-49 age category accounting for 15.00% (n=9). Those aged 70 and above made up a smaller fraction at 8.33% (n=5). When examining the age of menarche, it was noted that 36.67% (n=22) of the participants experienced menarche between 9 to 11 years, whereas a more sizable 63.33% (n=38) underwent menarche post the age of 11 years. Delving into the age at marriage, a significant 63.33% (n=38) were wedded between the ages of 15 to 18. Both the segments of participants who entered matrimony before 15 years and those who did so post 18 years held equal footing, each covering 18.33% (n=11) (Table 1).

Table 1: Demographic distribution of the study participants (N=60).

Variables	n	%
Age group		
40-49	9	15.00
50-59	29	48.33
60-69	17	28.33
≥70	5	8.33
Age of menarche (years)		
9-11	22	36.67
>11	38	63.33
Age of marriage		
<15	11	18.33
15-18	38	63.33
>18	11	18.33

It was observed that the highest Ki-67 labeling index was found 85 and the lowest was 45. Among the study cases, the mean of the Ki-67 labeling index was 58.32±9.27.

Table 2: Distribution of the study patients by Ki-67 labeling index, LI (N=60).

Variables	Mean±SD
Ki-67 LI	58.32±9.27
Range (min-max)	45-85

We observed that when the mean of Ki-67 labeling indices was compared with the different histopathological diagnoses of cervical cancer (according to WHO) the association was found statistically significant (Table 3).

It was observed that, among the 60 cases, 22 cases (36.7%) showed strong membranous intensity, 26 cases (51.6%) showed weak and homogenous (impaired) membranous intensity, 9 cases (15.1%) showed negative staining and 3(5%) cases did not show any staining for E- cadherin expression as it was mentioned earlier in the material and method chapter (Table 4).

In the present study, most of the cases were squamous cell carcinoma. According to WHO (2014), 80% of cases of cervical cancer are squamous cell carcinoma. Regarding E-cadherin expression in 60 cases of cervical cancer, was more intense in well-differentiated squamous cell carcinoma (14 cases) and showed weak, homogenous expression in 2 cases. In moderately differentiated squamous cell carcinoma 7 cases showed strong positivity and 18 cases showed 3+ weak and homogenous staining. However, poorly differentiated squamous cell carcinoma did not reveal any expression of e-cadherin in 3 cases, in contrast weak, homogenous (2+) to negative intensity in 6 cases. Only one case of adenosquamous carcinoma showed reduced or negative staining regarding e-cadherin. The difference was statistically significant in regards to the staining pattern of e-cadherin in six types of cervical carcinoma (Table 5).

Table 3: Association of mean Ki-67 of the study patients with histopathological diagnosis (N=60).

Histopathological diagnoses	Ki-67		P value
	Mean±SD	Min-max	
Moderately differentiated SCC	58.84±7.4	48-85	0.016[s]
Well differentiated SCC	55.19±9.54	45-75	
Poorly differentiated SCC	68.22±8.87	65-85	
Carcinoma in situ (CIS)	50.75±5.84	40-55	
Adenocarcinoma	59.2±11.8	45-75	
Adenosquamous carcinoma	65±0	65-65	

s=significant, p value reached from the ANOVA test

Table 4: Distribution of the study patients by degree of e-cadherin expression (N=60).

Degree of e-cadherin expression	Frequency	%
0, negative	3	5.0
1+ negative staining	9	15.1
2+weak and homogenous intensity	5	8.2
3+ weak and homogenous	21	35.0
4+ strong intensity	22	36.7

It demonstrates the comparison between the staining pattern of e-cadherin and the labeling index of Ki-67 regarding different types of cervical cancer and different grade among 60 cases. It shows that when there is a negative staining pattern of e-cadherin (in 3 cases) the mean labeling index of Ki-67 is higher, that is 71.0±7.9. And when E-cadherin expression is stronger in intensity the mean Ki-67 labeling index also gets lower, 52.5±8.2. Which has been proved to be statistically significant that has been obtained by the ANOVA test (Table 6).

Table 5: Association of degree of e-cadherin expression with histopathological diagnosis (N=60).

Degree of e-cadherin expression	Histopathological diagnosis					
	Well differentiated SCC	Moderately differentiated SCC	Poorly differentiated SCC	CIS	ADCA	AdSqca
0, negative	0	0	3	0	0	0
1+ negative staining	0	0	4	0	4	1
2+weak and homogenous intensity	0	0	2	3	0	0
3+ weak and homogenous	2	18	0	1	0	0
4+ strong intensity	14	7	0	0	1	0
P value	0.001s					

s=significant, p value reached from the Chi square test

Table 6: Comparison of Ki-67 labeling index and e-cadherin staining pattern in the different histopathological diagnoses of cervical cancer (N=60).

Degree of e-cadherin expression	Ki-67		P value
	Mean± SD	Min-max	
0, negative (n=3)	71.0±7.9	65.0-85.0	0.041[s]
1+ negative staining (n=9)	66.0±6.5	55.0-75.0	
2+weak and homogenous intensity (n=5)	60.2±10.8	47.0-70.0	
3+ weak and homogenous (n=21)	56.6±8.4	48.0-68.0	
4+ strong intensity (n=22)	52.5±8.2	45.0-82.0	

s=significant, p value reached from the ANOVA test

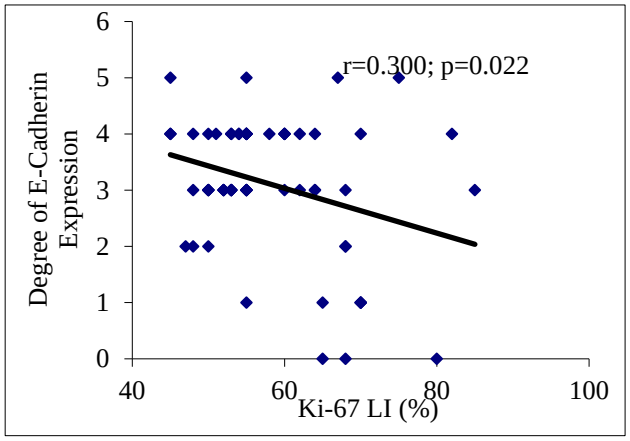

Figure 1: Scatter diagram showing negative significant correlation (r=-0.300; p=0.022) between Ki-67 LI (%) and degree of e-cadherin expression. (N=60).

It was observed that there is a negative, inverse but significant correlation between the expression of Ki-67 and e-cadherin which is statistically significant at the 0.05 level (Table 7).

Table 7: Correlation between expression of Ki-67 and e-cadherin in cervical cancer (N=60).

Correlations	Ki-67 LI (%)	Degree of e-cadherin expression
Ki-67 LI (%)		
Pearson correlation	1	-0.300*
Sig. (2-tailed)		0.020
N	60	60
Degree of e-cadherin expression		
Pearson correlation	-0.300*	1
Sig. (2-tailed)	0.020	
N	60	60

*Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

Within our exploration of the expression of e-cadherin and Ki-67 in cervical cancer, it's essential to emphasize the demographic composition of our study cohort. The age distribution distinctly highlighted the 50-59 age bracket as the foremost segment, constituting 48.33% (n=29) of the participants. This prevalence could offer insights into the age range where cervical cancer manifestations are most pronounced. The age of menarche, with a noticeable 63.33% (n=38) experiencing it post the age of 11 years, might have implications in understanding the early biological markers or risk factors associated with the disease. Additionally, the age of marriage, with a significant majority marrying between 15 to 18 years, could shed light on sociocultural factors that may play a role in disease prevalence or progression. Regarding the degree of e-cadherin expression, in this current study, 36.7% of cases showed strong intensity, 35.0%(3+) weak and homogenous, 8.3%(2+) weak and homogenous intensity, 15.1%(1+) negative staining and 5.0% had negative (0) which means no staining at all for E- cadherin expression. In Agnihotri et al study, e-cadherin showed a significant progressive loss of staining as the tumor differentiated from a well-differentiated grade to a poorly differentiated grade, where they found a significant progressive loss of staining as the tumor differentiated

from a well-differentiated grade to a poorly differentiated grade. Ninety percent of cases of well-differentiated, 80.0% of moderately differentiated while only 10.0% of poorly differentiated carcinomas showed 4+staining for E-cadherin.[12] Munhoz et al obtained in their study that positivity for e-cadherin in the cases diagnosed with CIN III was the lowest observed among the pre-neoplastic lesions at 55.96%, whereas, in cases of CIN, I was 82.18%, for CIN II, 77.39% and in the control group, 89.05%. There was a statistically significant difference when comparing the positive cases of invasive carcinoma 46.15% with CIN I and CIN II ($p<0.05$).[13] Ki-67, a proliferation marker known as a predictive factor for tumor development, is defined as a nuclear antigen (associated with hetero- and euchromatin) expressed during all active phases of the cell cycle (G1, S, G2, M) except G0; the level of Ki-67 expression is used to determine the cell proliferation status.[4,14] It can be detected through several qualitative and quantitative methods including monoclonal antibodies in immunohistochemical assays, electron microscopy, ELISA, flow cytometry, and immunocytochemistry.[2] The highest ki-67 labeling index found in this present study was 85 and the lowest was 45, as well as the mean of Ki-67 labeling index, was 58.32±9.27. One hundred and fifty-three cases of paraffin sections were studied by Ki67 immunostaining revealing an increased labelling index (LI) from dysplasia to carcinoma group.[15] This study showed the mean of the Ki-67 labelling index were compared with the different histopathological diagnosis of cervical cancer (according to WHO) the association were found statistically significant. The higher mean Ki-67 was found at 68.22±8.87 in poorly differentiated SCC, 65±0 in AdSqca, 59.2±11.8 in ADCA, 57.19±9.54 in WD SCC, 55.84±7.4 in moderately differentiated SCC and 52.75±7.84 in CIS. Munhoz et al. (2009) study found there was a statistically significant difference only between the median for the normal group 6.6% with the neoplastic lesions.[13] Invasive carcinomas 57.8%, was highly positive for Ki-67 when compared to CIN I 35.6%, CIN II 51.9%, and CIN III 40.9%, but there was no statistically significant difference was found between them ($p<0.05$). 53.8% of CC that have not survived was distributed in the mean Ki-67 proliferation group and 42.1% in the high proliferation group. Ki-67 proliferation marker is already recognized and validated as a specific and sensitive biomarker in cervical intraepithelial neoplasia.[16,17] It is well demonstrated that Ki-67 expression is increased in upper layers of cervical epithelia, being of major significance for the differentiation of non-neoplastic lesions that can mimic cancer.[4,18]

Moreover, the Ki-67 protein could be a biomarker of the proliferative activity and progressive potential of normal, dysplastic, and neoplastic cervical changes, with certain therapeutic implications.[4] Several studies have also suggested that Ki-67 may be a sensitive biological indicator of progression independent of age and menopausal status; Ki-67 can be used as an independent prognostic factor for the progression and biological behaviour of cervical dysplasia, especially when HPV infection assessment is missing.[17]

Limitations

The study was conducted in a single hospital with a small sample size. So, the results may not represent the whole community.

CONCLUSION

This study concluded that the degree of e-cadherin expression was significantly associated with Ki-67. There was a significantly negative correlation between Ki-67 LI (%) and the degree of E-Cadherin expression. Impairment of e-cadherin is very frequent in early-stage cervical cancers. So, it is recommended that both biomarkers are reliable for the diagnosis of cervical cancer. They can be used precisely to determine the exact grade of invasive cervical neoplasia and can be accepted as a diagnostic tool in malignant lesions of the uterine cervix.

Recommendations

This was a preliminary small group study to evaluate the proliferative index with Ki-67 and loss of immune-histochemical expression of E-cadherin which progress towards severity in cervical cancer. However, it is recommended to use these markers in all types of cervical carcinoma as part of routine pathological evaluations as per WHO's (2014) classification. Follow up or case-control study in a larger group should be included to see the further progression and for proper evaluation of patient.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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