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Original Article

E-Cadherin in Relation with the Proliferating Cell Nuclear Antigen of the Bilharzia Associated and Non-Associated Urinary Bladder Carcinoma

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Abstract

Background: E-cadherin is a trans-membrane glycoprotein that plays a critical role in many aspects of cell adhesion as well as establishment and maintenance of epithelial cell polarity. Loss of the adhesive function of E-cadherin seems to promote invasive and metastatic properties of neoplastic cells.

Objectives: The present study is a retrospective study aiming to evaluate the loss of E-cadherin immunohistochemical expression in relation with the proliferating cell nuclear antigen expression of the bilharzia associated and non-associated bladder carcinoma.

Methods: Forty TUR-bladder carcinoma sections immunohistochemicaly stained with E-cadherin antibody were microscopically interpreted and results were correlated to the established prognostic factors, including proliferating index as assessed by the proliferating cell nuclear antigen (PCNA) immunostaining, histopathological types, tumor cell grade, tumor invasiveness and bilharzia association.

Results: Histopathologically, 63% were transitional cell carcinoma, 33% were squamous cell carcinoma and 5% were adenocarcinoma. Loss of E-cadherin expression had a significant association with high PCNA index ($p<0.01$), the tumor grade ($p<0.002$), tumor invasiveness ($p<0.001$), and bilharzias-associated bladder cancer ($p<0.04$). There was no statistically significant association between loss or decrease of E-cadherin expression and histopathological typing of urinary bladder carcinoma ($p=0.094$).

Conclusion: Loss of E-cadherin provides an additional aid in assessment of prognosis and planning of therapy of patients with urinary bladder carcinoma as it indicates the potentiality for metastasis by its significant association with high proliferating cell nuclear antigen index, high tumor grade and tumor invasiveness. Moreover, immunohistochemical interpretation of E-cadherin altered adhesive function is a useful histological prognostic marker in bilharzia associated urinary bladder carcinoma.

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Keywords • E-cadherin • urinary bladder carcinoma • PCNA • bilhariziasis

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Introduction

Bladder cancer is a worldwide problem. It is linked to chemical agents as well as cigarette smoking. In the developing countries, it is linked to chronic infection with Schistosomiasis. Because of these links, bladder cancer has served as an excellent model for the study of cancer.[1] Bladder carcinomas account for 95% of all bladder tumors. carcinoma of the urinary bladder represents the forth most common malignancy in males and the tenth most common in females.[2] These tumors are one of the best-understood neoplasms with a well-defined etiology, natural history, tumor biology, treatment options and outcome. They arise as a consequence of multiple factors and represent a convergence of knowledge from diverse scientific disciplines, coupled with unique features of this neoplasm which make it amenable to detection, monitoring, treatment, and their combination makes this disease a model system for modern oncology.[3]

E-cadherin (E-Cad) is a member of a family of trans-membrane glycoprotein involved in intercellular adhesion and its function is mediated by interaction with the cytoplasmic alpha, beta and gamma catenins.[4,5] These catenins connect E-Cad with the cytoskeleton. E-Cad contributes to a variety of physiological functions like cell growth, differentiation, wound healing, cell motility, morphogenesis and organogenesis.[4,5] The loss of the adhesive function of the E-Cad is a critical step in progression of epithelial cells to a more malignant phenotype and also associated with tumor invasion and metastasis.[6,7] Decrease or loss of E-Cad might contribute to the malignant character of tumor cells and result in tumor progression.[8-10] E-Cad features a significant progressive loss of immunoreactivity in association with tumor dedifferentiation, advancing pathologic stage, and abnormal DNA content.[11,12] Abnormalities in the expression and cellular localization of E-Cad are frequently associated with high tumor grade, infiltrative growth, and lymph node metastasis in a variety of human malignancies.[4,13,14] This is a retrospective study aiming at exploring the expression of E-Cad protein in bilharzia and non-bilharzial bladder carcinomas in Egypt, and to relate the results of immunohistochemistry to the established prognostic factors, including proliferating index as assessed by the proliferating cell nuclear antigen (PCNA) immunostaining, histopathological types, tumor cell grade, tumor invasiveness and bilharzia association. Studies clearly show that altered adhesive function of tumor cells is important in the metastatic process and E-Cad is assumed to be critical in the malignant progression of many human tumors.

Materials and Methods

The current study included paraffin blocks of 40 transurethral (TUR)-bladder specimens of primary bladder carcinoma as well as five control specimens including 3 with cystitis and 2 normal urothelium. The routinely stained sections were re-examined microscopically and re-evaluated as regard to the histopathology and bilharzia infestation. Grading was performed according to the 1998 World Health Organization/International Society of Urologic Pathology grading system. Histologically the tumors were of low and high grades. Acording to Cheng *et al*, tumors are often under staged by TUR-bladder, although classification of low *vs.* high grades does predict the stage.[15] Histopathological interpretation of the tumor cell invasion confined to lamina propria included non-invasive types, invasive with muscle infiltration where tumor cells found were among bundles of smooth muscles.[16]

Immunohistochemistry

Formalin-fixed paraffin sections were processed, using a hot citric acid antigen retrieval method, for 30 min according to the manufacturer's recommendations and then incubated for 2 hrs with anti E-Cad and proliferating cell nuclear antigen (PCNA) monoclonal antibodies. The immunohistochemical staining was performed by labeled streptavidin-Biotin method with staining kit of Zymed Lab., Inc. USA. Following the instruction of the manufacture, the bound antibody complex was visualized by reaction in 3, 3' diaminobenzidine substrate and sections were counterstained with Meyer's hematoxylin. The staining intensities of E-Cad in tumor cells were evaluated compared with the adjacent normal urothelium as a positive control. A negative control slide was prepared by omission of the primary antibody. E-Cad immunostaining was classified into norm-expression; similar to normal urothelial E-Cad immunostaining, decreased expression, and loss of expression.[8] PCNA index was measured as the percentage of the number of the positively stained cells over the total number of the cells. Five high resolution fields from each slide were randomly chosen for the measurement.[17,18]

Table 1: Expression of E-cadherin immunoreactivity in relation to different prognostic pathological variables

Variables	E-cadherin Pattern						Total		P Value
	Normal Expression		Decreased Expression		Loss of Expression				
	no	%	no	%	no	%	no	%	
PCNA index									
<25%	1	2.5	8	20	1	2.5	10	25	<0.01
25-75%	1	2.5	7	17.5	6	15	14	35	<0.01
>75%	0	0	3	7.5	13	32.5	16	40	<0.01
Pathological type									
Transitional cell Carcinoma	2	5	8	20	16	40	25	62.5	NS
Squamous cell Carcinoma	0	0	9	22.5	4	10	13	32.5	NS
Adenocarcinoma	0	0	2	5	0	0	2	5	NS
Tumor grade									
Low grade	2	5	13	32.5	4	10	19	47.5	<0.002
High grade	0	0	5	12.5	16	40	21	52.5	<0.002
Tumor invasiveness									
Non-invasive	2	5	10	25	2	5	14	35	<0.002
Invasive	0	0	8	20	18	45	26	65	<0.002
Bilharzia association									
Non-Bilharzia	1	2.5	15	37.5	9	22.5	25	62.5	<0.05
Bilharzial	1	2.5	3	7.5	11	27.5	15	37.5	<0.05

Results

The current study included 40 TUR-bladder specimens with 25 (63%) transitional cell carcinoma, 13 (33%) squamous cell carcinoma, 2 (5%) adenocarcinoma and 15 (38%) bilharzia-associated bladder carcinoma. The study also included 5 control TUR-bladder specimens; 3 cases with cystitis and 2 normal urothelium for evaluation of normo-expression of E-Cad. Expression of the E-Cad protein was measured by staining the sections with specific E-Cad monoclonal antiserum. This antibody selectively stains normal epithelium, including urothelial epithelium in a characteristic membrane-specific pattern. The normal bladder mucosa samples, as well as normal urothelial epithelium adjacent to the neoplasm studied, showed strong uniform staining for E-Cad (Fig 1A).

E-cadherin in relation to PCNA
Loss of E-Cad expression had a significant association with high PCNA index ($p<0.01$). In those specimens with PCNA index of >75%, 13 (33%) had loss and 8 (20%) decreased expression of E-Cad. In those with PCNA index of 25%–75%, the loss of the E-Cad was seen in 6 (15%), and decreased expression of E-Cad was observed in 7 (18%) specimens.

E-cadherin in relation to histopathological type
There was no statistically significant association between loss or decrease of E-Cad expression and histopathological typing of urinary bladder carcinoma ($p=0.094$). Loss of E-Cad expression was mainly evident (Fig. 1B) in transitional cell carcinoma (TCC) in 16 cases (40%) and was decreased in 8 cases (20%). Decreased expression of E-cad was mainly observed (Fig 1C) in 9 cases of SCC (22.5%). Two patients (5%) with adenocarcinoma presented decreased E-Cad immunoreactivity (Fig. 1D).

E-cadherin in relation to tumor grade
The expression of E-Cad was significantly correlated with the tumor grade ($p<0.002$). Loss of E-Cad expression was observed in high-grade tumors (16 cases; 40%) whereas only 2 cases (30%) with low-grade tumor had decreased E-Cad expression.

E-cadherin in relation to tumor invasiveness
Expression of E-Cad significantly ($p<0.001$) correlated with tumor invasiveness of which 18 cases (45%) with invasive tumors presented with loss of E-Cad.

E-Cad in relation to bilharzia association
There was a significant association between loss or decrease of E-Cad expression and bilharzias-associated bladder cancer ($p<0.04$) (Table 1); 11 cases of bilharzia-associated bladder cancer (28%) presented with loss of E-Cad and 3 patients (8%) presented with decreased E-Cad expression. On the other hand, in those with non-bilharzia tumors, 9 (23%) had loss of E-Cad expression and 15 (38%) had decreased E-Cad expression.

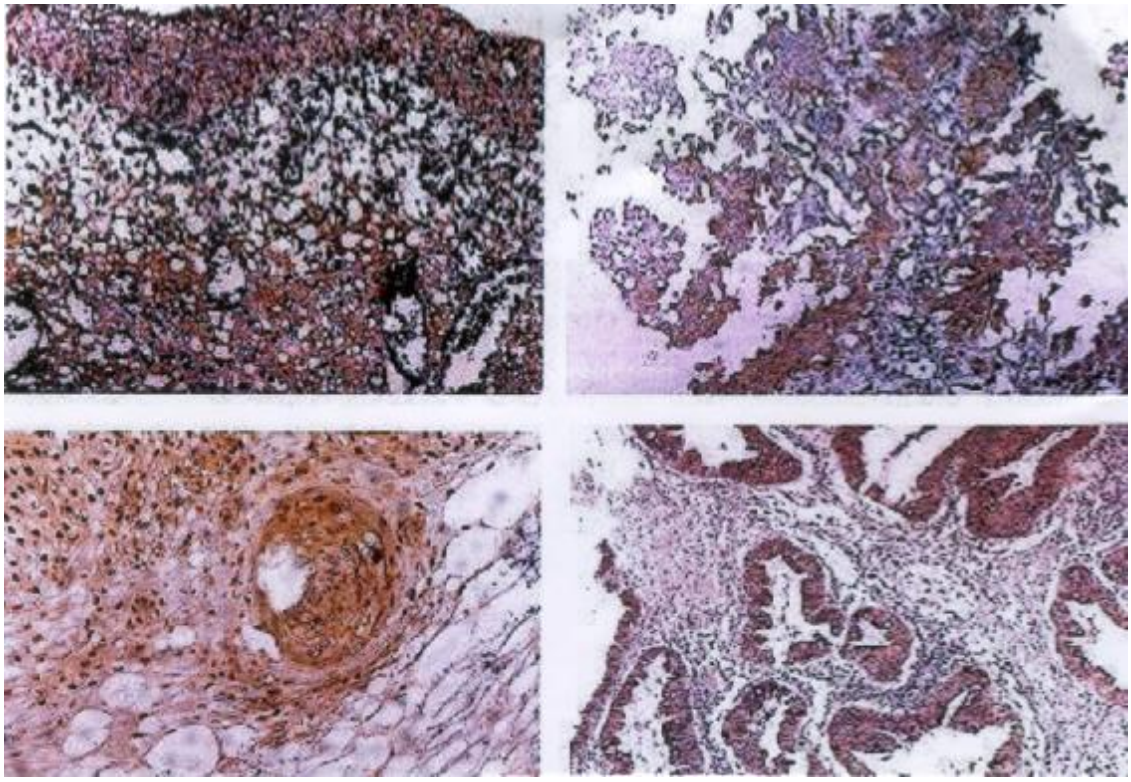

Fig 1: A (top left): Normal expression of E-cadherin in normal urothelium (X100). B (top right): Decreased E-cadherin immunostaining in transitional cell carcinoma (X200). C (bottom left): E-cadherin in squamous cell carcinoma (X200). D (bottom right): E-cadherin in adenocarcinoma (X200).

Discussion

Understanding the biology underlying tumorigenesis and tumor progression of urinary bladder cancer is essential for improving the capacity to diagnose and treat the disease.[2,19] The E-Cad as a trans-membrane glycoprotein modulates calcium-dependent intercellular adhesion in a variety of epithelial tissues. In many human cancers, including several forms of epithelial cancers, the level of E-Cad proteins is greatly reduced as compared to normal tissue, therefore, allowing dissociation of individual cells from tumor mass, meaning that E-Cad molecule is consistent with the function of the tumor suppressor gene and that the loss of its function seems to be involved in invasion and metastasis of neoplastic cells. Moreover; cell proliferation is a fundamental biologic defect in cancer.[20,21] Thus, assessment of the growth fraction provides a valuable prognostic index of the biological property of human neoplasm.[22,23]

To determine the potential prognostic value of the expression of epithelial E-Cad molecule, we analyzed its immunoreactivity in 40 urinary bladder carcinomas (TUR-bladder specimens) using an avidin-biotin immunoperoxidase technique on formalin-fixed paraffin-embedded tissues. E-Cad immunoreactivity pattern in urinary bladder carcinoma presented norm-expression in 5% compared with that seen in the normal urothelium. E-Cad is expressed homogeneously with a typical intense membranous staining at cell-cell borders, while decreased expression was detected in 45% and loss of expression in 50%.

E-Cad and PCNA play an important role in the tumor-genesis.[24] PCNA expression has been used to estimate the growth fraction of human cancer.[22,23] The present study evaluated the pattern of E-Cad expression and the status of PCNA expression as has been used to estimate the growth fraction of urinary bladder carcinoma. Loss of E-Cad expression presented a highly significant association with high PCNA index ($p<0.0007$). This finding was consistent with that of other reports.[23]

Abnormalities in the expression and cellular localization of E-Cad are frequently associated with high tumor grade, infiltrative growth, and lymph node metastasis in a variety of human malignancies.[7,25] This may explain the inverse relation between expression of E-Cad and tumor grade that was also found in certain

cancers.[26,27] In the present study, high grade tumors recorded loss of E-Cad expression in 40%, while 30% belonging to the low-grade tumor cell with decreased E-Cad expression. As regard to tumor invasiveness, 45% of invasive tumors presented loss of E-Cad. Statistically, loss of E-Cad expression presented a highly significant association with high tumor cell grade ($p<0.001$) as well as tumor cell invasiveness ($p<0.001$). The abnormal expression of E-Cad expression was significantly associated with the high-grade tumor cells and invasiveness of bladder carcinoma, as been reported in others studies too.[6,26,29,30]

Bilharzia associated bladder cancer presented loss of E-Cad (27.5%) and decreased of E-Cad expression (7.5%), as compared with 22.5% for loss of E-Cad expression and 37.5% for decreased E-Cad expression in the non-bilharzia tumors. There was a significant association between loss or decrease of E-Cad expression and bilharzia associated urinary bladder carcinoma ($p<0.04$). Studies reported that muscle invasion is the usual presentation of schistosoma-associated squamous cell carcinoma of the urinary bladder. It is unclear whether this invasive behavior is secondary to the aggressive nature of the disease or to delay in diagnosis.[31]

Rebel *et al*, reported that the loss of E-Cad by tumor cells is associated with increased tumor aggressiveness.[32] They demonstrated that E-Cad is an important determinant of the mechanisms which are involved in the recurrence rate of bladder cancer.[32] The high recurrence rate of human bladder cancer can be attributed to intra-epithelial expansion of tumor cells or shedding and subsequent implantation of tumor cells elsewhere in the bladder.[32] Moreover, Sánchez Cabayo suggested that the diagnosis and prognosis of bladder cancer would be enhanced by the use of markers, and that any marker may itself constitute a therapeutic target when studied in appropriate patients and control groups.[19]

Conclusion

Immunohistochemical interpretation of E-Cad is a useful histological prognostic marker in bilharzias-associated urinary bladder carcinoma. While, loss of E-Cad cellular adhesive function provides an additional aid in assessing the prognosis of patients with urinary bladder carcinoma as it indicates the potential of tumor cells to metastasize.

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