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ORIGINAL ARTICLE

E-Cadherin Expression on Urothelial Carcinoma and Its Association with Cancer Progression: A Retrospective Study

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ABSTRACT: ***Background:*** Bladder cancer is characterized by high recurrence and progressivity. E-cadherin serves as one of the most important molecules involved in the epithelial cells cell to cell adherence, suggested to inhibit tumor cells progression. This study aims to investigate the association between the E-cadherin expression with bladder cancer progressiveness in three years. ***Methods:*** This study was a retrospective cohort study involving bladder cancer patients. Diagnosis of bladder cancer was confirmed by histopathological and immunohistochemical examination with both grading and staging determined by histopathologist and oncologists. E- cadherin was examined through immunohistochemistry examination at the time of diagnosis. Data on demography, muscle invasion, clinical staging, grade, metastasis, multifocality and recurrence were obtained from medical records and pathology reports. The association of E-cadherin expression to muscle invasion and non-muscle invasion bladder cancer was evaluated and statistically analyzed. Patients' survival data were followed up by phone. ***Results:*** Forty bladder cancer patients with a mean age of 60.05 +/_ 10.3 years were included. Most subjects had high E- cadherin expression (85%), muscle invasion (65%), high grade (65%), no metastasis (87.5%), multifocality (65%) and no recurrence (62.5%). Lower expression of E- cadherin was associated with the higher clinical stage ($p<0.02$) and metastasis($p<0.001$). Patients with low E-cadherin expression showed worse cumulative survival than the higher one (mean 32 months vs 25 months, $p= 0.13$). ***Conclusion:*** Low level of E-cadherin was associated with the higher risk of muscle invasion, clinical staging, histological grade and risk of metastasis. Meanwhile, patients with high level of E- cadherin showed a better 3-year survival rate.

Keywords: Bladder Cancer, E-Cadherin, Metastasis, Recurrence, Survival.

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INTRODUCTION

Bladder cancer ranks as the 10th most commonly occurring cancer and 1.9% of overall cancer mortality is caused by this cancer.[1] Bladder cancer is classified based on its molecular subtypes; bladder cancer is divided into luminal and basal tumors. E-cadherin, a protein which can be found in luminal tumors, is one of the most important molecules in the process of epithelial cell to cell adherence in human tissues. The reduction of E-cadherin expression causes the barrier made by the cell-to-cell adherence to be disrupted thus increasing the susceptibility of invasion and metastasis due to uncontrolled proliferation of the tumor cells. The understanding regarding the pathogenesis of bladder cancer invasion should be taken into consideration as the progression of Ta/T1 cases to more advanced cases. To avoid that many prognostic tools have been developed since 1973, for instance, the world health organization (WHO) grading system.[2, 3] WHO grading system was used to determine bladder cancer prediction of recurrence and progression for NMIBC. The 2004/2016 version outperformed the late grading. Another prognostic scoring system for predicting NMIBC progression is the risk tables published by European platform of cancer research (EORTC) Genito-urinary cancer group. The first cystoscopy after transurethral resection of bladder tumor (TURBT) has also been used to examine the recurrence and progression in Ta/T1/Tis tumors. Prognostic role of molecular markers has been also investigated but until now there is insignificant data. E-cadherin is one of the potential molecule markers to determine progressivity since its ability to maintain intercellular connection by adhesion. Cheng *et al.* showed E-cadherin was associated with cancer specific survival and a lower level of E-cadherin gave significantly worse progression free survival in NMIBC.[4] We aim to investigate the association between E-cadherin level expression and to the risk of bladder cancer progressivity, both in NMIBC and MIBC patients.

METHODS

Study design

The design of this study was a retrospective cohort with a total sampling of all patients with bladder cancer that had treatment in Mymensingh Medical College Hospital and private specialized hospital in Mymensingh, Bangladesh (a tertiary referral hospital). The subjects included in this study were treated bladder cancer patients who had undergone histopathological and immunohistochemistry evaluation in Mymensingh Medical College Hospital and private specialized hospital in Mymensingh, Bangladesh between 2015 to 2024. Staging of bladder cancer was evaluated by experienced histopathologist and oncologist accounting signs, symptoms and results from supportive modalities using the standard staging system by American Joint Committee for cancer (AJCC). Demographic and cancer characteristics data were obtained through medical records and pathology reports. All patients who underwent both histopathological and E- cadherin immunohistochemistry examination with complete medical record data were included in this study. All subjects were followed by phone 36 months after treatment to evaluate all-cause mortality. All data were recorded prior to this study. All methods were performed in accordance with the relevant guidelines and regulations.

Immunohistochemistry

Immunohistochemistry examination was done from bladder cancer formalin fixed paraffin embedded specimens, which were cut into four micrometer tissues. Antigen retrieval was done through heat induced epitope retrieval in PH6 using a pressure boiler 125 degree centigrade and cooled to 90 degrees centigrade. Endogenous peroxidase enzymes were blocked using H_2O_2 0.3% and ethanol 95%. E-cadherin antibody used was produced by Sigma Aldrich (St Louis, Missouri). Incubation was done in labelled polymer HRP followed by chromogenization in DAB (substrate DAB: chromogen DAB was 1:20) using DAKO stainer KIT. The specimens were then counterstained using hematoxylin. The examination was performed visually by two examiners and then confirmed by an experienced pathologist. The expression of the protein was considered positive if cells were positively stained with brownish color on the cell membrane of the epithelial cells. A 3+mark were given to cells with full-thickness circumferential expression of the E-cadherin, a 2+mark was given to cells with full-thickness non-circumferential expression of the E-cadherin, 1+mark was given to cells with a faint expression of the E-cadherin and a 0 mark was given to cells without any visible expression of E-cadherin. Each cell assigned to different mark groups was counted and processed into a specific formula: [1 × (%cells + 1) + 2 × (%cells + 2) + 3 × (%cells + 3)]. The H-score ranged from 0 to 300, depending on the expression of E-cadherin. Further grouping for E-cadherin levels was done by defining low E-cadherin levels as a score below 100, as published by Loh *et al*., Corso *et al*., and Balamurugan *et al*.,.[5-7]

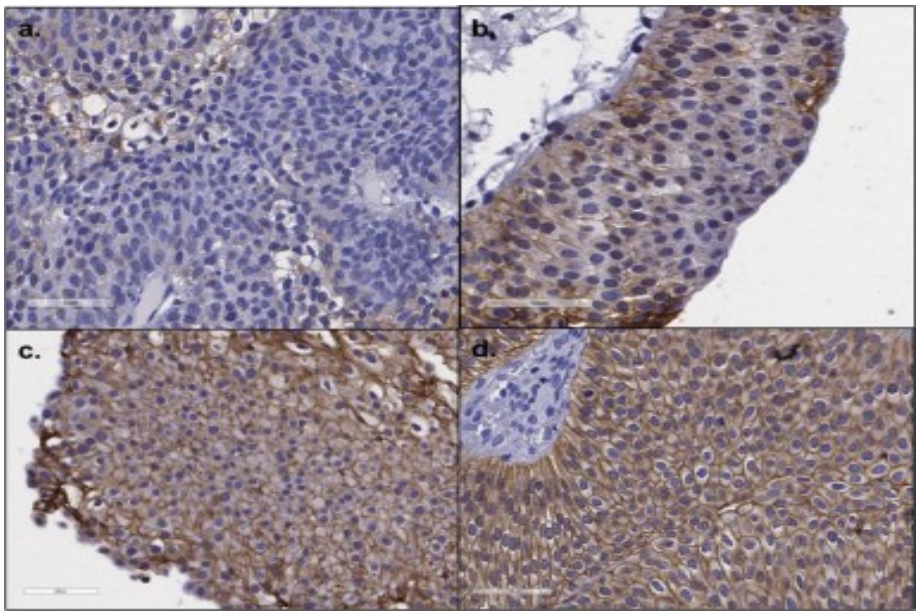

Figure 1: Immunohistochemistry of Antibody-Stained FFPE Bladder Cancer

Immunohistochemistry of antibody-stained FFPE bladder cancer preparation for E-cadherin was done per specimen counting minimum of 1000 cells using Aperio Image scope (Leica Bio systems, Bufalo Grove, IL, USA); **a.** field dominant with 0 expression mark (×400 magnification); **b.** field dominant with+1 expression mark (×400); **c.** field dominant with+2 expression mark (×400); **d.** field dominant with+3 expression mark (×400).

Statistical Analysis

All collected data were analyzed using IBM SPSS version 25. For 2 × 2 bivariate analysis, Fisher's exact test was done with a 95% confidence interval (p-value<0.05). For more than 2 × 2 table, Kruskal–Wallis test was done. Survival analysis based on E-cadherin category was done by Kaplan–Meier test with log-rank.

RESULTS

After evaluating the eligibility of the patients, 40 subjects were included in the study. The mean age of subjects was 10.3±60.05 years and 31 of the subjects were male. Mean H-score of bladder cancers was 177.15 (95% CI 157.88–196.42). After 36 months, 17 subjects were lost to follow up, so the data of all-cause mortality was incomplete. Data of bivariate statistical analysis of E-cadherin expression and each outcome were shown in Table 1. None of NMIBC patients were found in low E-cadherin levels and the majority of MIBC patients had high E-cadherin levels. Only stage and metastasis had statistically significant difference with E-cadherin. The relative risk (RR) for metastasis based on E-cadherin expression was 2 3.03–169.53).

A log-rank test was run to determine if there were differences in the survival distribution for the E-cadherin expression. The survival distributions for the high and low E-cadherin expression were different at cumulative survival analysis, even though it was not statistically significantly different (mean 32 months vs 25 months, p=0.13). Subgroup analysis of survival based on muscle invasion and E-cadherin expression was done. Mean survival time of subjects with MIBC with low E-cadherin, MIBC with high E-cadherin, and NMIBC with high E-cadherin were 25.2 months (95% CI 16.3– 34.1 months), 32.2 months (95% CI 26.1–38.2 months) and 32 months (95% CI 24.7–39.2 months), respectively (p=0.24) (Table 2 and Fig. 2).

DISCUSSION

Our study showed that lower expression of E-cadherin was associated with the higher clinical staging as well as higher number of metastases.[8] E-cadherin, one of the members of the cadherin family, plays a vital role in cell-to cell adhesion. It is a basic part of the adherence junction. Its role as tumor suppressor has been recognized. Without E-cadherin, cells can grow on top of each other, leading to initial formation of cancer.[9] Tis pathophysiology justified the role of low E-cadherin in cancer progression.

Earlier studies showed that lower E-cadherin expression contributed to higher number of MIBC and high histological cancer grades.[10, 11] Those findings showed the same result as the study conducted by Ying *et al.,*.[12] All NMIBC cases in our study had high E-cadherin expression, corresponding to other studies.[13, 14] Interestingly, some of the high-grade and MIBC cases retained their E-cadherin expression. This finding suggested the phenomenon of E-cadherin mutation as a factor of cancer progression, other than loss of expression. Mutated E-cadherin is detected in an immunohistochemistry assay but no longer had its original function in cell adhesion and tumor suppression.[15] Some cancers may also exhibit the ability to down regulate E-cadherin adhesive activity through an unknown mechanism.[15] Both mutated and down regulated E-cadherin retain their expression, which may explain why some cancers with high E-cadherin expression progress to MIBC. E-cadherin was not statistically associated with multifocality, but data showed high E-cadherin resulted in the existence of multifocality (70.6% vs. 29.4%) than low E-cadherin which the majority found in no multifocality cases (66.7% vs. 33.3%). Current literature showed that multifocal bladder cancer may arise from two sources: divisions of single cell (clonogenic) and simultaneous multiple transformations (field changes). In theory, clonogenic multifocality may need loss of cell adhesion (represented by E-cadherin), while field changes do not. Both hypotheses were still the subject for extensive studies.[16, 17] The other explanation is the mutated or down regulated expressed E-cadherin, as discussed above.[15] However, we found no research that specifically studied.

Table 1: Association of E-cadherin expression and outcomes (Muscle invasion, stage, grade, metastasis, multifocality, recurrence and mortality)

Outcomes	E-Cadherin		P
	High (n, %)	Low (n, %)	
Muscle Invasion			
NMIBC	14(100)	0(0)	0.06
MIBC	20(76.92)	6(23.08)	
Stage			
I	13(100)	0(0)	0.02
II	7(87.5)	1(12.5)	
III	7(100)	0(0)	
IV	7(58.3)	5(41.67)	
Grade			
Low	14(100)	0(0)	0.06
High	20(76.92)	6(23.08)	
Metastasis			
No	33(94.29)	2(5.71)	0.001
Yes	1(20)	4(80)	
Multifocality			
No	10(71.43)	4(28.57)	0.1
Yes	24(92.31)	2(7.69)	
Recurrence			

No	21(84)	4(16)	0.6
Yes	13(86.67)	2(13.33)	
Mortality			
No	13(86.67)	2(13.33)	0.29
Yes	5(62.5)	3(37.5)	

P Value of less than 0.05 was accepted as statistically significant. Mortality was recorded in only 23 patients, while the rest were lost to follow up.

Table 2: Kaplan-Meier Survival Table of Subjects in Three Years Based on Muscle Invasion And E-Cadherin Expression

	Time to event (months)	Number of Patients died at the time	Probability of survivors at the end of time
MIBC			
High E-Cadherin	5	1	0.91(±0.09)
	21	1	0.82(±0.12)
	24	1	0.73(±0.13)
	26	1	0.64(±0.14)
Low E-cadherin	9	1	0.8(±0.18)
	21	1	0.6(±0.22)
	24	1	0.4(±0.22)
NMIBC			
High E-Cadherin	8	1	0.86(±0.13)

* None of NMIBC subjects had low E-cadherin

The E-cadherin group showed only 40% probability of surviving beyond 36 months, while the high E-cadherin group showed around 70% subjects survived to 36 months. Furthermore, in subgroup analysis, subjects with MIBC had lower probability of survival (64% in high E-cadherin and 40% in low E-cadherin) compared to NMIBC (86%) within three years. The lack of statistical association was perhaps due to a loss of follow-up in 17 (42.5%) of subjects. As discussed earlier, E-cadherin holds a significant role in cell-to-cell adhesion.[15] With low E-cadherin, there is a higher risk for cancers to progress and metastasize.[18] This result would be comparable to the differences of expression found in the different staging and grading of bladder cancer which also translate into the tumor's aggressiveness and subsequently, the patients' prognosis. Therefore, E-cadherin expression had the potential to become a prognostic factor in MIBC and NMIBC. There were several limitations of our study. First, the sample size of our study was limited. Next, our follow-up data was incomplete. Because our hospital was a national referral hospital with patients originated across the division, we encountered difficulties in tracking the subjects after three years. Third, the type of treatments received by the subjects were unaccounted in our study, which could lead to outcome bias. Other limitations of this study were unaccounted co-founding factors, such as obesity, diabetes mellitus, chronic lung disease and congestive heart failure, which may aggravate the recurrence and survival rate. Those co-founding factors were difficult to adjust because of the rarity of the disease and single-centered nature of our study. Nevertheless, we conducted this study because E-cadherin is an important prognostic marker and scarcely studied in our country.

CONCLUSIONS

In our study, a low level of E-cadherin was associated with higher clinical staging and risk of metastasis. It also has the tendency to be associated with muscle invasion and histological grade. Low E-cadherin expression also has the tendencies to worsen survival and maybe potential to predict patients' prognosis.

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