E-Cadherin Expression in Transitional Cell Carcinoma of the Urinary Bladder and its Correlation with Histopathological Grade and Tumour Stage

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Abstract

Background: Urinary bladder cancer is nearly three times more common in men with male to female ratio of 4:1. The aim of this study was to detect, analyze the frequency and pattern of E-cadherin expression in morphologically diagnosed transitional cell carcinoma of the urinary bladder in relation to their grade and TNM staging.

Methods: This descriptive cross sectional study was carried out at the Department of Pathology, Dhaka Medical College.

Results: Among 50 cases of Transitional cell carcinoma of the Urinary bladder for histopathologic study of whom male to female ratio was found 4:1. Mean age of the patients were 59.30 ± 10.43. Majority of the patients (41.2 %) were low grade in 61-70 years and (36.4%) were high grade in 51-60 years of age. All patients were presented with anaemia and hematuria. There are 56.0% are histologically reported as high grade and 44.0% as low grade urothelial carcinoma according to WHO grading. Negative value of E-cadherin expression is significantly higher in high grade tumour (P<0.05). Majority of the metastasis cases are E-cadherin negative. Significant association was observed between the expressions of E-cadherin (P value <0.001) of the cases with different grading and staging of the tumours of urothelial carcinoma. Negative E-cadherin expression was observed in 24 high grades UC and 4 low grades UBC. Positive E-cadherin expression were observed in 9 high grade and 13 low grade UBC.

Conclusion: E-cadherin expression association with tumour grading and staging can help in predicting the clinical outcome. They have also therapeutic application and help in selecting patients for biological therapy.

Introduction

Urinary bladder cancer (UBC) ranks ninth in worldwide cancer incidence. It is more frequent in men than in women.[1] It is the fourth most common cancer in male and the eighth in female with male to female ratio 3:1.[1] The rate of incidence of bladder cancer increases with age.[2] Transitional cell carcinoma represents about 90% of all bladder tumors. Though the prevalence is more in developed countries, but now the incidence is increasing in developing countries like Bangladesh due to industrialization and smoking and betel nut habit, expose to chemical carcinogen and easy availability of relative investigation.[3]

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The exact incidence in Bangladesh is not well known. National Cancer Control Strategy and Plan of Action 2009-15 shows UBC is one of that 10 most common causes registered for treatment.[4]

The pathological staging of UBC is usually performed after surgical resection of the primary tumour and followed by cystectomy. It depends on pathologic documentation of the anatomic extent of the tumour. NMIBC (Non muscle invasive bladder cancer) is a heterogeneous disease by a high rate of recurrence rate by 15–61% at one year, depending upon risk category. Progression to MIBC (Muscle invasive bladder cancer) is also a concern for high-risk NMIBC patients, occurring in up to 17% of patients in one year.[5]

However, the overall prognosis is good with 65–85% of patients surviving for 5 years or more. Progression to or presentation with MIBC represents the critical step in the disease course, requiring more radical therapies and carrying a 5-year survival rate of only 25–50%. For treatment purpose, MIBC are treated by neo-adjuvant chemotherapy followed by radical cystectomy.[6]

Prognosis of UBC depends on tumour grade, stage, lymph node involvement, patient's age, location of tumour, vascular invasion, tumour infiltrating lymphocytes, P53 over expression, loss of E-cadherin etc. Generally, the higher grade & advanced stage has poor prognosis. The outcome can be different in patients at the same pathological grading and staging.[7] Prognostic factors are insufficient in determination of the outcome of disease.[8] Although, tumour grades and stages are conventional clinicopathologic parameters and are known to be prognostic factors for urothelial carcinoma, there are also many controversial cases. Several biological and molecular parameters have been suggested as potential prognostic markers for bladder cancer. Accurate prognosis with any single factor is difficult to predict. Other than this, the therapeutic weapons are limited in urothelial carcinoma of bladder and they permit only a limited improvement.

Epithelial cadherin (E-Cad) is a transmembrane glycoprotein and it is main component in desmosomes and adherence junctions.[9] Loss of E-cadherin function leads to more aggressive and invasive phenotype in many human cancer.[10]

Recurrence is common in all patients with non-muscle invasive urothelial cancer and high grade tumours. Loss of E-cadherin expression causes separation of the cells from cohesive epithelial tissues and leads to un-differentiation and invasiveness in a group of solid tumours, showing the important role of E-cadherin as a suppressor for malignant cells invasion and metastasis.[11]

Though the histological grade and tumour stage determines the most important prognostic variables for tumour progression, but they cannot predict accurately the behaviour of most bladder tumors. In Bangladesh, study of E-cadherin expression in Transitional cell carcinoma has not been evaluated properly. Adhesion molecule like E-cadherin can there by guide the oncologist for selection of those patients who are high risk for cancer recurrence and progression of bladder cancer. Therefore, they may be benefited from adjuvent treatment modality or targeted therapeutic strategy and the eventual use of neo adjuvant therapy in developing countries like ours.

The aim of the study was to observe the pattern of expression of E-cadherin in invasive and noninvasive UBC and their correlation with the WHO 2004 Grading and TNM staging system

Methods

This was a descriptive cross-sectional study. This study was carried out at the Department of Pathology, Dhaka Medical College over a period of two years from January 2016 to December 2017. A total of 50 cases were enrolled in this study. Patients of any age group with histologically diagnosed as Transitional carcinoma of the urinary bladder were included and Patient who received neo-adjuvant or adjuvant chemotherapy, massive necrosis and cautery effects were excluded in this study. During the collection of specimen, all relevant information's were recorded systematically in a prepared proforma. All the cases were numbered chronologically and the same number was given to histological as well as in immunohistochemically slides. To do this study ethical clearance was taken from institutional ethical committee of Dhaka Medical College. Bladder tumours were sampled or removed with biopsy instrument. All obtained specimens were immersed in 10% buffered formalin. After that proper H&E and immunostaining were done according to standard protocol. The histopathological report of bladder tumour biopsy contained grade, configuration (papillary or solid), and depth of penetration, presence of muscle, lymphatic invasion, blood vessel invasion, changes in the adjacent mucosa if present.[12] Immunostaining for E-cadherin was done at Square Hospital, Dhaka. For immunohistochemistry staining 4-micrometer thick tissue sections were taken on Poly-L lysine coated slide from the paraffin blocks of tumour. The presence of E-Cadherin staining in epithelial cells of normal urinary bladder mucosa in cystectomy specimen served as an internal positive control. Evaluation of immunostaining and scoring was performed by light microscopy. Only the membrane staining intensity and pattern was evaluated on a scale of 0 to 3.

Score 3: Membranous immunoreactivity, means surface expression of E-cadherin in >90% of the urothelial cells stained positively with high density)
Score 2: Heterogenous staining (i.e.<90% positive tumour cells)
Score 1: Cytoplasmic only
Score 0: Negative or absence of staining, excessively considerable fraction of E-cadherin negative cells.

For statistical analysis purposes, tumours were divided in two groups:
First group: With the score 03(normal group, membranous expression of E-cadherin)
Second group: Tumour specimens with score 0,1 and 2 (heterogenous, cytoplasmic, and negative staining).
Scores of 0, 1+ and 2+ were considered negative. Immunoreactivity of 3+ was scored as positive. Cytoplasmic staining was rare, considered nonspecific, and not included in assessment.[13] All necessary and relevant data were processes. Data were evaluated by standard statistical methods. Analysis was done by SPSS (Statistical Package for Social Science) by applying appropriate formula.

Results

Maximum (60.0%) patients were below or equal to 60 years and 40.0% patients were more than 60 years old. Mean age of the patients were 59.30 ± 10.43. Male were predominant. Maximum patients had habit of smoking and betel nut chewing.

Table I: Demographic profile of the patients

	Frequency	Percentage
Age (years)		
≤ 60	30	60.0
>60	20	40.0
Mean ± SD (Min-Max)	59.30 ± 10.43 (34 - 80)	
Gender		
Male	39	78.0
Female	11	22.0
Personal history		
Smoking	36	72.0
Betel nut chewing	36	72.0

Among the 50 patients, 56.0% are histologically reported as high grade and 44.0% as low grade urothelial carcinoma according to WHO grading.

There was significant difference between extent and histologic tumour grading (P <0.05). Distribution of patients according to its extent in low and high grade tumour (n=50)

Table II: Shows distribution of patients according to extent of tumour

Extent of tumour	Low grade (n=17)	High grade (n=33)	Total	p-value
T1	15 (88.2)	7 (21.2)	22 (44.0)	<0.001
T2	2 (11.8)	22 (66.7)	24 (48.0)	
T3	0 (0.0)	3 (9.1)	3 (6.0)	
T4	0 (0.0)	1 (3.0)	1 (2.0)	

Chi-square test was done to measure the level of significance.
Figures in the parenthesis denote corresponding %.

Table III: Negative value of E-cadherin expression is significantly higher in high grade tumour (P<0.05)

E-cadherin expression	Histopathology grade			p-value
	Low grade (n=17)	High grade (n=33)	Total	
Positive	13 (76.5)	9 (27.3)	22 (44.0)	0.001[s]
Negative	4 (23.5)	24 (72.7)	28 (56.0)	

[a]Chi-square test was done to measure the level of significance, s= significant
Figures in the parenthesis denote corresponding %.

Table IV: Shows there was no significant difference in E-cadherin expression among PT1, PT2, PT3 and PT4 with low and high grade tumour (P>0.05). Extent of tumour in E-cadherin expression positive and negative cases in low and high grade tumour (n=50)

Extent of tumour	Low grade			High grade		
	Positive	Negative	p-value	Positive	Negative	p-value
T1	13 (100.0)	2 (50.0)	0.044[b]	3 (33.3)	4 (16.7)	0.502[a]
T2	0 (0.0)	2 (50.0)		6 (66.7)	16 (66.7)	
T3	-	-		0 (0.0)	3 (12.5)	
T4	-	-		0 (0.0)	1 (4.2)	
Total	12 (100.0)	5 (100.0)		22 (100.0)	14 (100.0)	

[a]Chi-square test was done to measure the level of significance.
[b]Fisher's Exact test was done to measure the level of significance.
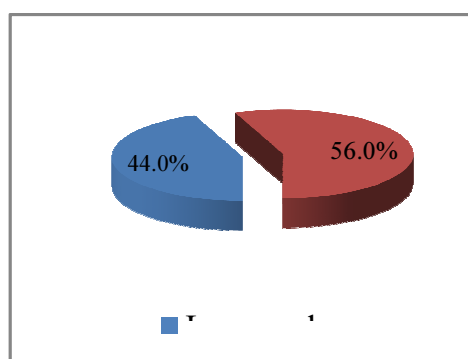Figures in the parenthesis denote corresponding %.

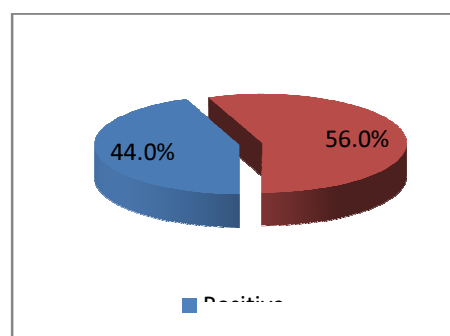Figure 1. Pie chart showing the tumour grading of study patient.

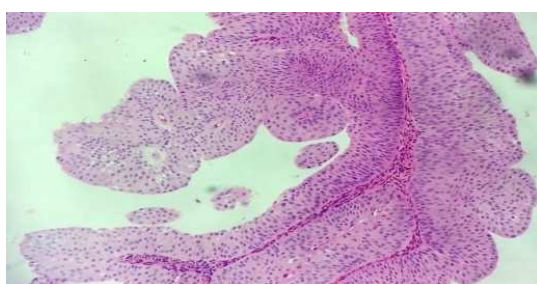Figure 2. Among the 50 patients, 56.0% are E-cadherin negative and 44.0% are positive.

Fig 3. Photomicrography showing histological section of Low grade papillary urothelial carcinoma (Case no: 1064, H&E x400)
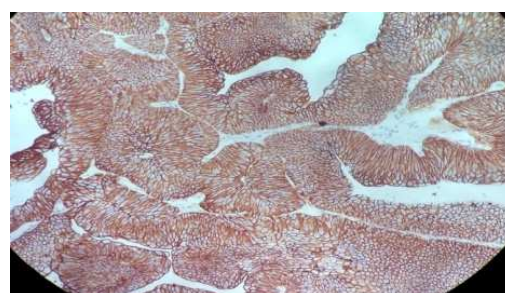

Fig 4. Photomicrography showing Positive E-cadherin immunostain in low grade papillary urothelial carcinoma(Case no: 1064/4015, H&E x400)
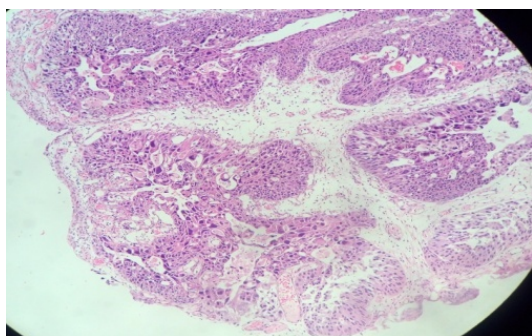
Fig 5. Photomicrography showing High grade urothelial carcinoma, PT3 (Case no: 3083, H&E x100)
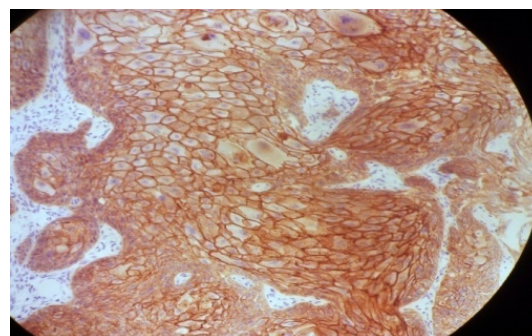

Fig 6. Photomicrography showing Positive E-cadherin immunostain (Score 3+ staining) in high grade urothelial carcinoma (Case no: 1379/4028, H&E x400)

Discussion

In current study, mean age of the patients was 59.30 ± 10.43 (34 - 80) with male to female ratio was found 4:1 that is similar to the study of Jawad, Ali and Kamal (2016).[14] They found mean age of the patients was 58.72±1.6 with male was 73.3% and female was 26.7%. A similar study by Hossain, 2010 in BSMMU, Dhaka, found most of the patient with bladder cancer to be over 50 years of age.[15] Men had a threefold greater risk of developing bladder cancer than women.[16] The higher incidence of UBC in male may be due to the personal habit such as smoking and more exposed to toxic agents due to their occupation.

In this present study 72% patients had habit of smoking and betel nut chewing. Chinnasamy et al., (2016) revealed most of bladder cancer patients (71.2%) had smoking habit which was consistent with this study result.[17] Chou et al., (2013) found 24.9% of urothelial cancer patients had smoking habit.[18]

Regarding clinical findings and investigation, all (100%) the cases of this study presented with hematuria. Similar finding was observed by Chinnasamy et al. (2016).[17]

It was observed that 56.0% patients had high grade urothelial carcinoma (HGUC) and 44.0% patients had low grade urothelial carcinoma (LGUC). Chou et al., (2013) in their study found 56.8% high grade and 43.2% low grade tumour. Incidence of high grade UC patient was more in our study. [18] In our country the probable cause may be poor economic condition, lack of knowledge, lack of urological treatment facilities as well as social and religious restrictions especially for female patients which prevent them from utilizing hospital facilities.

As we had taken PT3 and PT4 specimen so histology related to lymph node status was available.

In this present study, the extension of tumour was assessed in all 50 specimens (35 cases of TURBT and 15 cases of radical cystectomy). In the 17 low grade urothelial carcinoma, 15(88.2%) had showed tumour extension up to stage PT1 and 2(11.8%) up to PT2. In 33 high grade urothelial carcinoma it was 7 (21.2%) PT1, 22(66.7%) PT2, 3(9.1%) PT3, 1(3.0%) PT4. Zaman, 2012 in BSMMU, Dhaka observed 10% low grade and 48% high grade cancer were extension up to PT1 in his study [19] and in India Chinnasamy et al., (2016) found PT1 86.5% and PT2 13.5%.[17] Without an adequate resection and good

quality of underlying detrusor muscle, the pathologist will not be able to fully differentiate between Ta, T1 and T2 bladder cancer.[20] But in our study, it was possible to detect stages of tumour upto stage PT4.

E-cadherin is known to be the prime mediator of intercellular cohesion and epithelial tissue integrity, these alterations may lead to loss of cell differentiation and allow cells to detach from the primary site. [21] In present study, abnormal staining patterns (negative, heterogeneous or cytoplasmic only) were seen frequently (56%) in bladder cancer.

Of the total 33 high grade Urothelial Carcinoma, E-cadherin expression was found to be positive in 27.3 % cases, 72.7 % were negative (score 0, 1, 2). There are 13 (76.5%) positive E-cadherin was observed in low-grade and 9 (27.3%) negative E-cadherin in high grade. It was also revealed that 4 (23.5%) negative in low grade of 17 patients and 24 (72.7%) negative in high grade of 33 patients. The difference was statistically significant (p<0.001) with the histologic grading of UBC. Syrigos et al., (1995) found near similar to these findings.[22]

Correlation with tumour stage category showed that 22 of 50 T1+T2 stages (22/50, 44.0%) showed normal E-cadherin expression comparing to no patients with T3+T4 stages. Membranous E-cadherin was also most frequently lost in bladder cancer patients of T3 and T4 tumour category (4/4, 100%) compared to 20/29 (68.9 %) of T1+T2 stage tumour category. K. Charalabopoulos et al., (2003) found 17 of 40 Ta+T1+T2 stages (17/40, 42.5%) showed normal E-cad expression comparing to only 1/5 (20%) patients with T3+T4 stages.[14] Similar findings also seen in Syrigos et al., (1995).[22]

In this study, we found significant correlation of E-cadherin expression between the grading

of bladder tumour and TNM staging. These results were supported by some other studies done by K. Charalabopoulos et al., (2003); Syrigos et al., (1995),[22] Khorrami et al., (2012).[23]

Low grade with positive E-cadherin expression are associated with decreased aggressiveness and required lower aggressive therapy. There were 4 cases seen in our study, which were morphologically low grade but showed negative E-cadherin expression. Low grade UBC with negative expression of E-cadherin are associated with increased biological aggressiveness and more metastatic disease. This is supported by Khorrami et al., (2012).[23] In this present study, we observed 28(56.0%) E-cadherin negative high grade UC cases. They will be considered as high risk patients with bladder cancer and will need more aggressive therapy.

In this current study, we observed 4(100%) E-cadherin negative in PT3+PT4 and 24 (48.0%) in PT1+PT2 UC cases. Negative cases will be considered as high risk patients with bladder cancer and will need more aggressive therapy. There were 09 high grades UC patients with positive expression of E-cadherin found in this study. These are the controversial cases. Other prognostic factors should be taken into consideration such as lymph node status, staging, gene amplification etc. for their treatment.

Conclusion
The expression profile of the biomarkers may be useful for the selecting high risk patients with bladder cancer for proper treatment. Hence patients who have a low risk of recurrence, need to identify in order to avoid over treatment as well as those who likely to progress in order to treat them more aggressively. In Bangladesh no study was conducted in E-cadherin expression in associated with urothelial carcinoma. This

study could have been more effective if more number of urothelial carcinoma cases were included and follow up was done to see the progression of the disease and recurrence. Work is in progress to establish whether normal membranous E-cadherin expression can be enhanced by gene therapy or biological therapy to induce a less invasive and metastatic phenotype.

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