Expression of E-Cadherin in Malignant Epithelial Neoplasms of the Urinary Bladder

[1]Hany Khattab, [2]Hosni K. Salem, [1]Mostafa Salem, [1]Mostafa Khodeir and [1]Maya Elserafy

[1]Department of Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt
[2]Departement of Urosurergy, Faculty of Medicine, Cairo University, Cairo, Egypt

Abstract: Globally, bladder cancer is one of the most common malignancies and a major cause of cancer morbidity and mortality. Loss of E-cadherin expression is considered a critical factor in facilitating the progression of bladder cancer, The aim of this work is to study the immunohistochemical expression of E-cadherin in malignant epithelial neoplasms of the urinary bladder and then possible prognostic values. 52 radical cystectomy specimens; collected from the Pathology department, Faculty of medicine, Cairo University, during the period from September 2011 up to December 2012. The two 4-µm thick sections were cut from the formalin fixed, paraffin embedded tumor blocks.One was stained with H&E for histopathological reassessment and one was stained immunohistochemically by E-cadherin monoclonal antibody The immunohistochemical demonstration of the immune markers was accomplished by using immunoperoxidase method. Results showed that loss of E-cadherin expression was found to be significantly correlated with high stages of urinary bladder carcinoma and positive lymph nodal metastasis. Also, a significant correlation was detected between E-cad expression and the histological type of the tumors. In conclusions, the application of E-cad immunohistochemical marker could be used as an independent predictor of lymph node metastasis.

Key words: Urinary Bladder Carcinoma · Immunohistochemistry · E-Cadherin · TCC · SCC · Schistosoma Infestation

INTRODUCTION

Globally, bladder cancer is one of the most common malignancies and a major cause of cancer morbidity and mortality [1]. In developing countries particularly in the Middle East and Africa, the majority of bladder cancers are squamous cell carcinomas (SCCs) and most of these cancers are secondary to Schistosoma haematobium infection. In Africa, the highest incidence of SCC has been seen in schistosomal-endemic areas, notably Sudan and Egypt, where SCC ranges from two thirds to three quarters of all malignant tumors of the bladder. Few studies from Egypt have shown a reversal of this trend due to better control of schistosomiasis in the region, whereas in other parts of Africa the association is unchanged [2, 3]. The major prognostic factors in carcinoma of the bladder are the depth of invasion into the bladder wall and the degree of differentiation of the tumor. Most superficial tumors are well differentiated. Patients in whom superficial tumors are less differentiated,

large, multiple, or associated with carcinoma in situ in other areas of the bladder mucosa are at greater risk for recurrence and the development of invasive cancer [4]. The cell-cell adhesion protein E-cadherin represents a cell surface glycoprotein which accounts for a Ca-dependent homotypic adhesion. It plays a role in organization and maintenance of tissue structure [5].

Lack of E-cadherin expression may lead to diminished cellular adhesion, resulting in invasion into connective tissue and possibly to metastasis [6]. In bladder cancer, altered E-cadherin expression is associated with the degree of invasiveness, lymph node metastasis and increased risk of death from bladder cancer [7]. Loss of E-cadherin expression is considered a critical factor in facilitating the progression of bladder cancer [8].

Therefore, we conducted this study to assess the immunohistochemical expression of E-cadherin in malignant epithelial neoplasms of the urinary bladder and the possible prognostic values.

Corresponding Author: Maya Elserafy, Department of Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt.
E-mail: ma_mohey@yahoo.com

MATERIALS AND METHODS

Study Design: This is a retrospective cross sectional study. The material of this study consists of 52 radical cystectomy specimens of human urinary bladder carcinomas. All specimens were collected from the Pathology department, Faculty of medicine, Cairo University. The period of recruitment was from September 2011 up to December 2012. All the specimens were radical cystectomy specimens with pelvic lymphadenectomy. The clinical data of these patients including age and sex were taken from their pathology requisition sheets enclosed with the specimens.

Histopathological Evaluation: Hematoxylin and Eosin stained slides were evaluated for the histological type of the tumor according to World Health Organization (WHO) histological classification of tumors of the urinary tract; 2004 and for tumor grade based on AJCC/UICC TNM, 7th edition; 2010. Other architectural features were assessed as the extent of invasion, presence of vascular and/or nerve invasion, lymph node metastases and associated schistosomal infestation [9].

Immunohistochemical Staining: Sections were mounted on already coated glass slides. The slides were incubated at 37°C overnight for accurate adhesion of the section to the slide, deparaffinized by incubation in the oven at 56°C for 15 minutes and inserted in xylene for 30 minutes, rehydrated by transferring into graded ethanol, then washed in phosphate buffer saline (PBS) (pH 7.2) for 5 minutes. Slides were immersed in a solution of 90 ml methanol + 10 ml hydrogen peroxide (1.5%) for 30 minutes for blocking of endogenous peroxidase. Slides were placed in citrate buffer, then heated in a microwave oven at 100°C for three successive times, five minutes

each for antigen retrieval. Tissue sections were covered immediately with 2 drops of blocking reagent (normal rabbit serum) and were left for 30 minutes in humidified chamber to suppress non-specific binding of immunoglobulins. The rabbit monoclonal anti- E-cadherin antibody of IgG type is applied to detect endogenous levels of total E-cadherin protein (Isotype: Rabbit IgG, Molecular weight: 135 KDa, #3195, stored at -20°C, dilution 1:400) (manufactured by Cell Signaling Technology, Inc.). Application of link antibody [biotinylated goat secondary anti-polyvalent {anti-mouse IgG (H+L) and anti-rabbit IgG (H+L)} catalog # TP-015-BN], then application of labeling reagent streptavidin-horseradish peroxidase complex (Catalog # TS-015-HR) is done. The antigen was finally localized by the addition of DAB chromogen (3-3\ diaminobenzidine tetrahyrochloride) plus substrate (Catalog # TA-015-HSX) and DAB plus chromogen (Catalog # TA-001-HCX). Slides were immersed in Mayer's hematoxylin for 3-5 minutes. Slides were placed in 2 changes of 95% ethanol, then 2 changes of absolute alcohol for rehydration. The sections were washed in between the successive steps by distilled water and by PBS. In each staining session, an internal control of normal urothelium was used as positive control for E-cadherin. As a negative control, a tumor tissue section was processed in the above mentioned sequence but the primary antibody was not added and instead (PBS) was used in this step. The membranous expression of E-cadherin (at the cell-cell border) immunostaining was evaluated according to Elzagheid *et al*. [10] as follows: (2+) more than 40% of tumor cell membranes showed staining. (1+) from 10 to 40% of tumor cell membranes showed staining. (-) less than 10% of tumor cell membranes showed staining Figure (1).
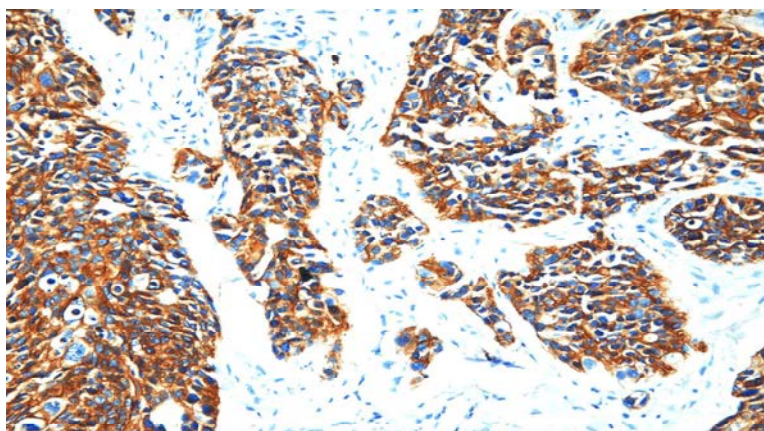

Fig 1: Micrograph of low grade urothelial carcinoma, E-cadherin immunostained, score 2 +, (magnification x 200).

Statistical Methods: All analyses were done using SPSS (Statistical Package for Social Sciences) software, version 18, Chicago, IL, USA. Categorical variables were expressed as frequencies and percentages. Chi-square test was used for testing proportion independence and Fisher Exact tests if expected number of observations in 25% or more of the cells is less than 5. Kappa measured concordance of markers positivity and negativity being perfect if equal to1.0. P value was set significant if ≤ 0.05 level.

RESULTS

The mean age of the cases was 60 years old, with minimum of 40 years and maximum of 80 years. Most of our cases were males (87% of all cases), with male: female ratio of 6.4:1. Most of the tumors (79%) were more than 3 cm in diameter. Seventy three percent of cases have TCC and 63% of cases were high grade. Most of TCC cases had high grade tumors (76%), while most of SCC cases had moderately differentiated tumors (71%). Most of our cases were pT 3 stage which represented 46% of all cases and tumors with extravesical mass or extension

(pT3b and pT4) represented three fourth of all our cases (75% of cases). One third of our cases had lymph nodal metastasis. Thirty eight percent of our cases showed positive vascular invasion in their examined specimens. Forty two percent of our cases had Bilharzial ova in their specimens. We found a significant correlation between SCC tumors of the urinary bladder and Schistosomal infestation (71% of SCC cases were infested, in comparison to only 32% of TCC cases). Eighty one percent of all cases (42 cases out of 52 cases) showed positive E-cadherin expression.

There is no significant correlation between E-cadherin expression and either the age, sex of the patients or size of the tumors. We detected positive E-cadherin expression in 100% of SCC cases, while in only 74% of TCC cases. This correlation proved to be statistically significant. 24% of patients having high grade tumors showed loss of E-cad expression, while 11% of patients having low grade tumors showed loss of expression of the marker. Also there is a significant correlation between decreased E-cadherin expression and high stages of urinary bladder carcinoma.100% of tumors having pT stage less than 3b (tumors confined to

Table 1: Demographic and histopahtological results of the studied groups

		E-cad Negative	E-cad Positive	P value
Age	≤50 y	2(4%)	5(10%)	0.500
	>50 y	8(15%)	37(71%)	
Sex	Female	2 (4%)	5 (10 %)	0.500
	Male	8 (15 %)	37 (71%)	
Size of the tumour	≤3 cm	3	8	0.446
	>3 cm	7	34	
Histological Type	SCC	0(0%)	14(27%)	0.046
	TCC	10(19%)	28(54%)	
Grade	Low	2	17	0.293
	High	8	25	
pT Stage	2a	0(0%)	4(7.5%)	0.033
	2b	0(0%)	5(10%)	
	3a	0(0%)	4(7.5%)	
	3b	9(17%)	11(21%)	
	4a	1(2%)	15(29%)	
	4b	0(0%)	3(6%)	
T stage	Negative (≤3a)	0(0%)	13(25%)	0.050
	Positive (>3a)	10(19%)	29(56%)	
Pelvic organ invasion (T4)	Negative	9(17%)	24(46%)	0.050
	Positive	1(2%)	18(35%)	
LN	Negative	4(7%)	31(60%)	0.041
	Positive	6(12%)	11(21%)	
Vascular invasion	Negative	7(13%)	25(48%)	0.541
	Positive	3(6%)	17(33%)	
Schistosomal infestation	Negative	6	24	1
	Positive	4	18	

the bladder) positively expressed E-cadherin, while only 74% of cases with pT stage more than 3a (tumors grossly invading extravesical tissue or pelvic organs) showed positive expression of the marker in a significant correlation. There is a significant correlation between positive E-cadherin expression and tumors with pT 4. This might be due to the fact that 90% of cases having E-cadherin negative tumors were in the category of stage pT 3 while only one case out of nine was in the category of stage pT 4.

Decreased E-cadherin expression correlates with positive lymph nodal metastasis. It showed that about 90% of cases with no LN metastasis showed positive E- cadherin expression, while only 65% of cases having LN metastasis showed positive expression. No significant correlation between vascular invasion by tumor emboli in our included specimens and E-cadherin expression.

DISCUSSION

In our present work, most of the tumors in our specimens were transitional cell carcinoma (73%), while the rest were squamous cell carcinoma. The age of our 52 patients ranged between 40 and 80 years with the mean age 59.8 ± 7.4 years which was in concordance with other reports from Egypt, that found that the mean age of bladder cancer cases was 58.34 ± 12.13 years [11] and 59.0 ± 9.9 [12]. Our results were also near to those of Salem and Mahfouz, who detected an increase of the mean patient age from 41 ± 11.2 years to 52 ± 8.6 years [13] and Gouda *et al*. [3] who reported an increase in the median age of patients from 47.4 years to 60.5 years. Most of our cases were males (87%), with male to female ratio 6.4:1. This is near to the results observed by El-Bolkainy *et al* and Zarzour *et al*, where the male to female ratio was 5:1 and 5.5:1 respectively [11,14]. The male predominance is probably attributed to the fact that males are more exposed to the established risk factors, including bilharzial infestation, cigarette smoking and occupational exposure to chemical carcinogens [15, 16]. In discordance to our results, Salem and Mahfouz, reported a decrease in the male to female ratio which changed from 5.6:1 to 4.2:1 [13]. This difference may be due to larger sample size and the relatively longer duration of the study.

Millan-Rodriguez *et al*. [17] and Rodriguez-Alonso *et al*. [18] recorded that tumors more than 3 cm were associated with a greater risk of stage progression and reduced survival. This is also observed in our current study, as most of the tumors (79%) were more than 3 cm in diameter. At the same time, 75% of the tumors were high stages and showed extravesical mass or extension (pT3b and pT4).

In our study, about three fourth of the cases had TCC (73% of all cases) and only 27% of cases had SCC. This is almost similar to the results of Salem and Mahfouz, who found that a significant increase occurred in the prevalence of transitional cell carcinoma from 20% to 66%, with a significant decrease in the prevalence of squamous cell carcinoma from 73% to 25% [13]. In concordance, Ashley *et al*. [19] also detected that the relative frequency of TCC in their multi-year sample (26 years) increased from 22% in 1980 to 73% of bladder tumor diagnoses in 2005, while SCC decreased from 78% of diagnosed bladder tumors in 1980 to 27% of diagnosed bladder tumors in 2005. It also goes with the results of Gouda *et al*, who reported a significant decline of the prevalence of SCC from 75.9% to 28.4% and a significant rise in TCC from 16% to 65.8% of bladder cancers [3]. This might be attributed to better control and treatment programs of Schistosomiasis in Egypt especially in the rural population which was traditionally one of the most important risk factors of SCC in the urinary bladder. This was accompanied by a change in the histological profile of tumors, with significant predominance of transitional cell carcinoma and an increase in the age of patients, a pattern rather similar to that in western reports [3]. Also, increased smoking habits are believed to have contributed to the shift toward TCC in Egypt, which has a stronger smoking association [20].

Despite the use of advanced imaging techniques, regional or distant metastases may go unnoticed until surgery or at follow-up. There is ample documentation that clinical staging is inaccurate in one third of cases, with a tendency for underestimation of the extent of the disease. Therefore, there is a need for additional objective information on the aggressiveness of bladder tumors [23]. This is suggested by our results as most of our cases were pT3b (38% of all cases) and pT4a (31% of all cases). This means that many cases were understaged before cystectomy.

Our results showed that 38% of our cases show positive vascular invasion in examined specimens. This is quite near to the results of Thalita dos Reis *et al*. [22] who reported microvascular invasion in 50% of their examined specimens.

In relation to schistosomiasis-associated carcinoma of the urinary bladder, it was found that it represented a distinct clinico-pathological entity different from nonbilharzial carcinoma and it showed a high frequency

of squamous cell carcinoma due to squamous metaplasia and dysplasia of the transitional epithelium. Recently, however, a relative increase in the frequency of transitional cell type in schistosomiasis-associated bladder cancer has been noted [2, 24].

Our results revealed that 42% of our cases showed Bilharzial ova in their specimens. This agrees with the results of Salem and Mahfouz, who found that the incidence of associated Bilharziasis in bladder carcinoma decreased from 80% to 50% [13]. This also goes with the results of Gouda *et al*, who reported a significant decline of Bilharzial association in bladder carcinoma from 82.4% to 55.3% [3].

In our present study, 71% of SCC cases had Schistosomal ova in their specimens, while only 32% of TCC cases were infested. Statistical analysis showed a significant correlation between SCC tumors of the urinary bladder and Schistosomal infestation (P value= 0.01). In the same context, Rambau *et al*. [26] noticed that Schistosomiasis was commonly associated with squamous carcinoma compared to non squamous carcinomas. The relatively high frequency of bladder cancer in Egypt supports the etiological relationship to urinary schistosomiasis. Despite the marked decrease in prevalence of endemic schistosomiasis over the last 2 decades, Egypt is paying the toll of the previously high prevalence of the disease. Comparison of the frequency of active urinary schistosomiasis previously reported during the era of high prevalence of the disease and the age-specific incidence rate indicates a strong cohort effect Ibrahim and Khaled [26] and Jemal *et al*. [1], reported that the majority of bladder carcinomas associated with schistosomiasis are squamous cell carcinoma. Smoking and occupational exposures are the major risk factors in Western countries, whereas chronic infection with Schistosoma hematobium in developing countries, particularly in Africa and the Middle East, accounts for about 50% of the total burden.

Radical cystectomy is commonly the treatment of choice for patients with muscularis propria-invasive urothelial carcinoma. Unfortunately, up to 40% of patients whose disease is confined to the bladder wall will develop tumor recurrence; virtually all of them will die as a result of the disease. Thus, it is imperative that we find additional markers (clinical, pathologic, or molecular) to predict adverse clinical events in these patients [27].

Cadherin/catenin-based anchoring junctions organize and connect the microfilaments to maintain cell adhesive properties and integrate intra- and intercellular signaling, which includes regulation of nuclear functions and transcription pathways [28, 29].

Reduction in expression of cell adhesion molecules has been reported in human cancers [30]. Decreased E-cadherin expression is involved in the initial step of invasion, which involve deregulation of cell adhesiveness resulting in tumor cell detachment and dedifferentiation in human carcinomas. Therefore, the investigation of E-cadherin in cancerous tissue, using immunohistochemical technique on tissue sections, may enable early prediction of the potential of cancer cells to detach from the parent tumor and their potential aggressiveness [31, 32]. In addition, loss of E-cadherin expression is a critical factor in facilitating the progression of bladder cancer [33].

In this study, about one fifth of our cases (10 cases out of 52 cases) showed loss of E-cadherin expression. This is quite near to that observed by Szekely *et al*. [34] who found that E-cadherin expression was lost in 13/40 (32%) of their bladder carcinoma cases. It also keeps with Shariat *et al*. [35] who detected loss of normal membrane E-cadherin immunoreactivity in 17 patients with bladder carcinoma (32%). These slight differences might be due to different geographical locations and different scoring systems used.

In our study, no significant correlation was found between E-cadherin expression and either the age or the sex of the patients. This is supported by the results of Szekely *et al*. [34] In concordance, Thalita dos Reis *et al*., reported that the correlation between E-cadherin immunohistochemical expression and the sex of the patients was insignificant [22].

In our study, no significant correlation was detected between E-cadherin expression and the size of the tumors (p value = 0.4). Parallel to this finding, in a study conducted by Hu *et al*. [36] the abnormal expression rate of E-cadherin in bladder cancer tissues was not significantly correlated with tumor size (p value = 0.6). Thalita dosReis *et al*. [22] also did not detect any significant association between between E-cadherin expression and the size of the tumors (p value = 0.3).

In this work, 24% of patients having high grade tumors showed loss of E-cad expression, while only 11% of patients having low grade tumors showed loss of expression of the marker, but the difference did not reach statistical significance. In the study conducted by Szekely *et al*. [34]they reported that there was no association between E-cadherin expression and tumor grade, age or gender of the patient. This is parallel to our results in these criteria. Similarly, Thalita dosReis *et al*. [22] detected insignificant relationship between E-cadherin expression and tumor grade.

Furthermore, Koksai *et al.* [37] also showed that abnormal E-cadherin expression had no association with the grade. Mialhe *et al.* [38]observed that E-cadherin expression was not correlated with the tumor grade, although it was highly correlated with the tumor stage. On the other hand, Hu *et al*. [36] reported that the abnormal expression rate of E-cadherin in bladder cancer tissues of the 72 cases included was significantly correlated with tumor pathological grade. Also, Jang *et al.* [39] detected that membranous E-cadherin expression was more decreased in tumors with high grade. This discordance may be much attributed to larger sample size used by these authors and different immunoreactivity cut-offs.

In our study, we found a significant correlation between decreased E-cadherin expression and high stages of urinary bladder carcinoma (P value < 0.009). In concordance to our results, Hu *et al*. [36] reported that the abnormal expression rate of E-cadherin in bladder cancer tissues was significantly correlated with tumor stage (P < 0.05).

Moreover, Clairotte *et al.* [39] observed a highly significant correlation between the decreased expression of E-cadherin and increased TNM stage (P value < 0.001). This is also keeping with the results of Koksai *et al.* [37] who reported that abnormal E-cadherin expression was associated with advanced tumor stage in their cases. This goes with the results of other studies by Byrne *et al*. [7] and Mialhe *et al.* [38] who observed that reduced expression of E-cadherin was associated with increased depth of invasion. On the other hand, Szekely *et al*. [34] reported no association between E-cadherin expression and tumor stage. This discordance can be explained as they conducted their study on urinary bladder carcinoma having lower pathological stages in TUR specimens.

In our present study, we detected a statistically significant correlation between decreased E-cadherin expression and positive lymph nodal metastasis (P value= 0.041). In the study done by Byrne *et al.* [7] they concluded that, in bladder cancer, altered E-cadherin expression was associated with lymph node metastasis (p value= 0.044). In addition, they demonstrated significant association between altered E-cadherin expression with the degree of invasiveness and increased risk of death from bladder cancer.

Also, in this study, a multivariate logistic regression analysis showed a significant correlation between loss of E-cad expression and lymph node metastasis (p value= 0.01). This means that application of E-cad immunohistochemical marker on small biopsy specimens

(TUR-T) could be used as an independent predictor of lymph node metastasis in a safe, easy and relatively affordable method. This should be further proved in multicentric studies with larger series of patients.

Several publications proved that the loss of E-cadherin expression is associated with poor prognosis, high tumor grade and stage, while others did not detect these associations. These differences might be explained by the fact that the loss of E-cadherin expression is associated with disturbances at different levels (mRNA or protein), or with disturbances of the expression of the E-cadherin-associated catenins [40]. This might explain why our results were concordant with some studies and discordant with others.

In our results, a significant correlation was found between pelvic organ invasion (stage pT 4) and positive E-cad expression. Our explanation to this is that 90% of our cases having negative E-cad expression were in the category of stage pT 3b while only one case out of nine was in the category of stage pT 4. Other studies with larger sample size or with different scoring systems might be able to solve this problem.

The determination of prognosis in bladder cancer is currently based on staging methods that depend primarily on the pathological stage of a tumor which is highly subjective. That's why we need to find more objective criteria for the determination of prognosis. Dysregulation in cell-cycle regulation markers is involved in the development and progression of bladder cancer. Individual alterations of these molecules are associated with the degree of aggressiveness of a given tumor and its impending outcome [41].

As far as we know, no other study noted the statistically significant correlation between E-cad immunohistochemical expression and the histological type of urinary bladder carcinoma (TCC versus SCC).

CONCLUSION

The use of E-cadherin in pathological specimens of urinary bladder carcinomas may have a prognostic value as it significantly correlated with each of the pathological stage of the tumors and the histological type of the tumors.

REFERENCES

1. Jemal, A., T. Murray, E. Ward, A. Samuels, R.C. Tiwari, A. Ghafoor, E.J. Feuer and M.J. Thun, 2005. Cancer statistics, 2005. CA Cancer J. Clin., 55: 10-30.

2. Heyns, C.F. and A. van der Merwe, 2008. Bladder cancer in Africa. Can J. Urol; 15(1): 3899-3908.

3. Gouda mE., N. Mokhtar, D. Bilal, T. El-Bolkainy and N.M. El-Bolkainy, 2007. Bilharziasis and Bladder Cancer: A Time Trend Analysis of 9843 Patients. Journal of the Egyptian Nat. Cancer Inst; 19(2): 158-162.

4. Lagwinski, N., A. Thomas and A. Stephenson *et al.,* 2007. Squamous cell carcinoma of the bladder: a clinicopathologic analysis of 45 cases. Am J Surg Pathol.; 31(12): 1777-87.

5. Takeichi, M., 1991. Cadherin cell-adhesion receptors as a morphogenetic regulator. Science; 251: 1451-1455.

6. Chen, V. and B. Obrink, 1991. Cell-cell contacts mediated by E cadherin (uvomorulin) restrict invasive behavior of L-cells. J. Cell. Biol.; 114: 319-327.

7. Byrne, R.R., S.F. Shariat, R. Brown M.W. Kattan, R.A.J.R. Morton, T.M. Wheeler and S.P. Lerner, 2001. E-cadherin immunostaining of bladder transitional cell carcinoma, carcinoma in situ and lymph node metastases with long-term follow up. J Urol.; 165(5): 1473-1479.

8. Imao, T., K. Koshida, Y. Endo T. Uchibayashi, T. Sasaki and M. Namiki, 1999. Dominant role of E-cadherin in the progression of bladder cancer. J Urol.; 161(2): 692-8.

9. Edge, S.B., D.R. Byrd, C.C. Compton, A.G. Fritz, F.L. Greene and A. Trotti, 2010. Urinary bladder. AJCC Cancer Staging Manual. 7th ed. New York, NY: Springer; pp: 497-505.

10. Elzagheid A., T. Kuopio, M. Ilmen and Y. Collan, 2002. Prognostication of invasive ductal breast cancer by quantification of E-cadherin immunostaining: The methodology and clinical relevance. Histopathology; 41(2): 127-33.

11. Zarzour, A.H., M. Selim, A.A. Hameed D.A. and M.A. Abdelaziz, 2008. Muscle invasive bladder cancer in Upper Egypt: the shift in risk factors and tumor characteristics. BMC Cancer; 8: 250.

12. El-Chennawi, F.A., F.A. Auf, S.S. Metwally, Y.M. Mosaad, A.A. Shaaban, M.A. El-Baz, Z.E. Tawhid and Z.F. Lotfy, 2009. Vascular endothelial growth factor, p53 and the H-ras oncogene in Egyptian patients with bladder cancer. World J Gastrointest Oncol; 1(1): 62-68.

13. Salem, H.K. and S. Mahfouz, 2012. Changing Patterns (Age, Incidence and Pathologic Types) of Schistosoma-associated Bladder Cancer in Egypt in the Past Decade. Urology; 79(2): 379-383.

14. El-Bolkainy, M.N., N.M. Mokhtar, M.A. Ghoneim and M.H. Hussein, 1981. The impact of schistosomiasis on the pathology of bladder carcinoma. Cancer; 48: 2643-2648.

15. El-Mawla, N.G., M.N. EL-Bolkainy and H.M. Khaled, 2001. Bladder cancer in Africa: update. Semin Oncol; 28: 174-178.

16. Gupta, P., M. Jain R. Kapoor K. Muruganandham, A. Srivastava and A. Mandhani, 2009. Impact of age and gender on the clinicopathological characteristics of bladder cancer. Indian J Urol; 25: 207-10.

17. Millan-Rodriguez, F., G. Chechile-Toniolo, J. Salvador-Bayarri, J. Palou and J. Vicente-Rodríguez, 2000. analysis of the prognostic factors of primary superficial bladder cancer.J Urol.; 163: 73-8.

18. Rodríguez-Alonso, A., S. Pita-Fernαndez and J. Gonzαlez-Carreró, 2002. p53 and ki67 expression as prognostic factors for cancer-related survival in stage T1 transitional cell bladder carcinoma. Eur Urol.; 41(2): 182-8.

19. Felix, A.S., A.S. Soliman, H. Khaled, M.S. Zaghloul, M. Banerjee, M. El-Baradie, M. El-Kalawy, A.A. Abd-Elsayed, K. Ismail, A. Hablas, I.A. Seifeldin, M. Ramadan and M.L. Wilson, 2008. The changing patterns of bladder cancer in Egypt over the past 26 years Cancer Causes Control.; 19: 421-429.

20. Jang, T.J.1., W.H. Cha and K.S. Lee, 2010. Reciprocal correlation between the expression of cyclooxygenase-2 and E-cadherin in human bladder transitional cell carcinomas. Virchows Arch.;457(3):319-28.Lagwinski,N., A. Thomas, A Stephenson. Campbell S, Hoschar AP, El-Gabry E, Dreicer R and Hansel DE., 2007. Squamous cell carcinoma of the bladder: a clinicopathologic analysis of 45 cases. Am J Surg Pathol.; 31(12): 1777-87.

21. Thalita dos Reis, S., K. Ramos Moreira Leite, A. Mosconi Neto, J. Pontes Júnior, N.I. Viana, A.A. Antunes, M.F. Dall'Oglio and M. Srougi, 2012. Immune expression of E-cadherin and α, β and γ-Catenin adhesion molecules and prognosis for upper urinary tract urothelial carcinomas. Int Braz J Urol; 38(4): 466-473.

22. Fernandez Mena, F.J. and Y.C. Moreno-Torres, 2001. Bladder cancer. Arch EspUrol; 54: 493-510.

23. Saied, C.M., W.H. El-Metenawy and M.S. Elwan, N.R. Dessouki, 2007. Urine carcinoembryonic antigen levels are more useful than serum levels for early detection of Bilharzial and non-Bilharzial urinary bladder carcinoma: Observations of 43 Egyptian cases. World J Surg Oncol.; 5: 4.

24. Rambau, P.F., P.L. Chalya and K. Jackson, 2013. Schistosomiasis and urinary bladder cancer in North Western Tanzania: a retrospective review of 185 patients. Infectious Agents and Cancer; 8: 19.

25. Ibrahim, A.S. and H.M. Khaled, 2006. Urinary Bladder Cancer. Cancer Incidence in Four Member Countries (Cyprus, Egypt, Israel and Jordan) of the Middle East Cancer Consortium (MECC) Compared with US SEER National Cancer Institute, NIH Pub. No. 06-5873.

26. Reuter, V., 2005. Lymphovascular Invasion as an independent predictor of recurrence and survival in node-negative bladder cancer remains to be proven. J Clin Oncol; 23: 6450-1.

27. Gumbiner, B.M., 2005. Regulation of cadherin-mediated adhesion in morphogenesis. Nature Reviews Molecular Cell Biology.; 6(8): 622-634.

28. Kurokawa, H. and M. Ikura, 2006. Perspectives on structure elucidation of the cadherin-catenin complex. Seikagaku; 78(7): 595-600.

29. Ghadimi, B.M., J. Behrens, I. Hoffmann W. Haensch, W. Birchmeier and P.M. Schlag, 1999. Immunohistological analysis of E-cadherin, alpha-, beta- and gamma-catenin expression in colorectal cancer: implications for cell adhesion and signaling. EurJCancer; 35: 60-65.

30. Rahman, N., J. Stone, G. Coleman, B. Gusterson, S. Seal, A. Marossy, S.R. Lakhani, A. Ward, A. Nash, A. McKinna, R. A'Hern, M.R. Stratton and R.S. Houlston, 2000. Lobular carcinoma in situ of the breast is not caused by constitutional mutations in the E-cadherin gene. Br. J. Cancer; 82: 568-570.

31. Turashvili, G.1., J. Bouchal, G. Burkadze and Z. Kolαr, 2005. Differentiation of tumors of ductal and lobular origin. Biomed. Papers; 149(1): 57-62.

32. Tian, X., Z. Liu, B. Niu, J. Zhang, T.K. Tan, S.R. Lee, Y. Zhao, D.C. Harris and G. Zheng, 2011. E-Cadherin/β-Catenin Complex and the Epithelial Barrier Int J Urol.; pp: 1442-2042.

33. Szekely, E., V. Torok, T. Szekely, P. Riesz and I. Romics, 2006. E-Cadherin Expression in Transitional Cell Carcinomas. Pathology Oncology Research, 12(2): 73-77.

34. Shariat, S.F., S. Pahlavan, A.G. Baseman, R.M. Brown, A.E. Green, T.M. Wheeler and S.P. Lerner, 2001. E-cadherin expression predicts clinical outcome in carcinoma in situ of the urinary bladder. Urology.; 57(1): 60-5.

35. Hu, X., Y. Ruan, F. Cheng, W. Yu, X. Zhang and S. Larré, 2011. p130Cas, E-cadherin and β-catenin in human transitional cell carcinoma of the bladder: Expression and clinicopathological significance. Int J Urol.; 18(9): 630-7.

36. Koksai, I.T., A.M. Ates, A. Danisman, C. Sezer, A. Ciftcioglu, G. Karpuzoglu and M. Sevuk, 0000. Reduced E-cadherin and α-catenin expressions have no prognostic role in bladder carcinoma. Pathol Oncol Res.; 12: 13-19.

37. Mialhe, A., J. Lois and S. Montlevier, 1997. Expression of E-cadherin and α-, β-, γ-catenins in human bladder carcinomas: Are they good prognostic factors? Invasion Metastases; 17: 124-137.

38. Clairotte, A., I. Lascombe, S. Fauconnet, F. Mauny, S. Félix, M.P. Algros, H. Bittard and B. Kantelip, 2006. Expression of E-Cadherin and α-, β-, γ-Catenins in Patients With Bladder Cancer Identification of γ-Catenin as a New Prognostic Marker of Neoplastic Progression in T1 Superficial Urothelial Tumors. Am J Clin Pathol.; 125: 119-126.

39. Bringuier, P.P., L.A. Giroldi, R. Umbas, T. Shimazui and J.A. Schalken, 1999. Mechanisms associated with abnormal E-cadherin immunoreactivity in human bladder tumors. Int J Cancer; 83: 591-595.

40. Mitra, A.P., D.E. Hansel and R..J. Cote, 2012. Prognostic value of cell-cycle regulation biomarkers in bladder cancer. Semin Oncol.; 39(5): 524-33.