Immunohistochemical Expression of Estrogen Receptor Beta in Urothelial Carcinoma of the Urinary Bladder

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Abstract

Background: The tenth most prevalent cancer type in the world is bladder cancer. Males are four times more likely than females to have it. The most prevalent subtype is urothelial carcinoma. Development and progression of bladder cancer are influenced by estrogen receptors.
Objectives: To examine the expression of estrogen receptor beta in bladder urothelial carcinoma and how it relates to other pathological factors of prognostic significance.
Materials and method: Fifty formalin fixed and paraffin-embedded tissue sections of cases of urothelial carcinoma from transurethral resection and radical cystectomy specimens were examined histopathologically by routine H&E stain and immunohistochemically stained with Anti-ER-beta antibody. The immunohistochemical expression of estrogen receptor beta was assessed.
Results: Of the 50 cases of urothelial carcinoma, mean age 60 ±8.7 range (41-85) years, 34 male and 16 female, estrogen receptor beta was expressed in 29 (58%) of the cases. The expression of ERβ was significantly elevated in high grade (p value 0.006). The ERβ expression was also significantly increased in advanced tumor stages (p value 0.03) and in muscle invasive tumor (p value 0.004). The presence of lymphovascular emboli was associated with higher ERβexpression.
Conclusion: ERβ expression is significantly associated with high grade and with advanced stages of urothelial carcinoma.
Keywords: Estrogen receptor beta; Immunohistochemistry; Urothelial carcinoma; Urinary bladder.

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Introduction

Bladder cancer is the tenth most common kind of cancer worldwide and it strikes males four times more often than women. It's the ninth leading cause of cancer death in males and the sixth most frequent malignancy **(Bray et al., 2018).** In Egypt, the bladder cancer was considered the 3rdcommon type of cancer (6.9%) after breast cancer and hepatocellular carcinoma **(Lotfy et al., 2021).**

Histologically, Urothelial carcinoma is the commonest subtype, which represent up to 90% of cases with bladder cancer in the world. It is due to chemical exposure, such as occupational exposure or tobacco smoke **(Saginala et al., 2020).**

Nearly 75% of bladder cancer cases fall under the category of non-muscle-invasive disease (Ta/is, T1), which is typically treated locally. The remaining 25% of cases are muscle-invasive (T2-T4), with cystectomy being the usual treatment method. Other options include transurethral resection, chemotherapy, and radiation therapy **(Cumberbatch et al., 2018).** According to the stage, grade and number of initial tumors, bladder malignancy recurrence occurs at a very high incidence of 50% to 90%, making lifetime surveillance for tumor recurrence imperative. **(George et al., 2013).**

By attaching to particular steroid receptors, sex steroid hormones regulate bladder cell carcinogenesis. Estrogen receptors are members of the nuclear receptor family, which may convert extracellular signals into a transcriptional response **(Shen et al., 2006).** Nuclear estrogen receptors come in two types in humans: ERα and ERβ. It has been shown that both types are present in bladder cancer cell lines and tissue, albeit ERβ is more common **(Ide and Miyamoto, 2021; Han et al., 2012).** Although they have the same binding affinity to the major estrogen, 17β-estradiol (E2), some ER agonists and antagonists preferentially attach to one (for example estrone/raloxifene to ERα) and they have different biological functions **(Ide and Miyamoto, 2021).**

Increasing evidence points to ER signaling, which is regulated by estrogen, as playing a key role in both the development and progression of urothelial cancer **(Goto and Miyamoto, 2021).** Higher ERβ function has been shown in multiple in vitro studies to boost bladder cancer cell proliferation, and ERβ signals have been shown to promote bladder cancer progression **(Miyamoto et al. 2012; Han et al. 2012).** In a preclinical model, 17 β-oestradiol treatment of mice with transplantable urothelial carcinoma led to tumor regression. Other studies shown that estrogens and particular ER modulators such tamoxifen and raloxifene, inhibited cell growth and induced death in bladder cancer lines that largely expressed the ERβ **(Miyamoto et al., 2012)**.

Materials and methods

Fifty tissue samples of urothelial bladder cancer that were formalin fixed and paraffin embedded (FFPE) underwent a retrospective study. These cases came from the pathology department archives at South Valley University's faculty of medicine. A total of 36 specimens were transurethral resection of the tumor (TURT), and 14 specimens of radical cystectomy. Data of the patients was retrieved from the referral sheets. Clinical

data including age and gender was identified.

Histopathological examination

Histopathological evaluation of Hematoxylin and Eosin (H&E) stained sections from the specimens were done and histopathological features were recorded including tumor type, the grade following the two tier WHO 2004/2016 of urothelial neoplasia **(Compérat et al., 2019)**, bladder tumor TNM stage as defined by the American Joint Committee (AJCC) (**Magers et al., 2019),** the presence of lymphovascular emboli or perineural invasion, lymph node involvement and the presence of bilharzial ova. The cases were further grouped into non-muscle invasive carcinoma (NMIC) which included stages pTa and pT1 and muscle invasive carcinoma (MI) which included stages pT2, pT3, and PT4.

Immunohistochemical staining

Immunohistochemical staining of (FFPE) tissue blocks were used to detect ER expression. These 4 micrometer sections were labeled and stained with ER using a Dako automated immunostainer and Dako visualization system (using prediluted, Mouse Anti-ER-beta antibody [ERb455]). The German Immunoreactive Score was utilized to evaluate and score the ER staining, which was identified as brownish nuclear staining. We multiplied the percentage of immunoreactive cells (0% = (0), 1% = (+1), 10% = (+2), 20% = (+3), and 80% = (+4)) by the staining intensity (negative +0, weak +1, moderate +2, and strong +3). Negative (0; 0-1), weak positive (+1; 2-4), moderate (+2; 6-8), and strong (+3; 9-12) were the categories assigned to scores (range 0–12) **(Miyamoto et al., 2012).**

Ethical considerations

The Ethics Committee of Faculty of Medicine, South Valley University in Qena, Egypt, has given its approval to the present research. **Code number:** SVU-MED-PAT005-1-21-3-170.

Statistical analysis

The data was analyzed using SPSS version 24. The numerical data was summarized as a mean SD. The qualitative information was presented as a frequency and percentage distribution. In order to determine statistical significance between two means, an independent-samples t-test was used. The Chi-Square test was developed to analyze statistical significance between discrete categories. p values less than 0.05 were deemed statistically significant.

Results

A total of fifty specimens of urothelial carcinoma were included in the study, mean age 60 ±8.7, age range 41-85 years. Thirty-four (68%) patients were male and 16 (32%) were females. The cases were diagnosed as; eighteen cases non-invasive, low-grade, papillary UC and two cases non-invasive, high-grade, papillary UC. Four cases invasive, low-grade, papillary UC (4T1) and three cases invasive, high -grade, papillary UC (2 T1,1 T2). Twenty-three cases were invasive, high -grade UC, five cases of them showed squamous differentiation, 1 micropapillary, 1 nested variant (12 T2, 6 T3, 5 T4). The histopathological features are illustrated in (**Table.1**).

Table1. Clinicopathological characteristics of the studied cases:

Variables	No. (n=50)	Percentage
Age		
Mean (SD)	60± 8.7	
Min- max	41-85	
Gender		
Male	34	68%
Female	16	32%
Tumor grade		
Low	22	44%
high	28	56%
Tumor stage		
Ta	20	40%
T1	6	12%
T2	13	26%
T3	6	12%
T4	5	10%
Muscle invasion		
NMI	26	52%
MI	24	48%
Tumor pattern		
Papillary	27	54%
Non papillary	23	46%
LN involvement		
Without	6	43%
With	8	57%
Lymphovascular invasion		
Absence	45	90%
Presence	5	10%
Perineural invasion		
Absence	48	96%
Presence	2	4%
Bilharziasis		
Absence	44	88%
Presence	6	12%

ERβ expression was positive in 29 (58%) out of 50 studied cases (9 are weakly positive, 12 cases are moderately positive, and 8 cases are strongly positive). There was no significant association between ERβ expression and the age or gender,(p-value=0.16, 0.161) (**Table. 2**).

In the fifty examined specimens, twenty-one (75%) (6 strong, 9 moderate, 6 weak) out of 28 high grade UC were ERβ positive while there is 8 (36.4%) (2 strong, 3 moderate, 3 weak) out of 22 low grade tumors were ERβ positive. ERβ expression was significantly associated with high grade compared to low grade UC (p value=0.006) (**Table .2** & **Fig. 1,2**).

Eighteen (78%) out of 23 non papillary UC were ERβ positive while 11 (41%) out of 27 papillary UC were ERβ positive. ERβ expression was associated with non-papillary pattern compared to papillary pattern UC (p value 0.007) (**Table .2** & **Fig.2**).

ERβ expression was significantly associated with tumor stage higher ERβ expression are found in more advanced stage (p value 0.03). ERβ was positive in 7/20 of pTa, 3/6 of pT1, 9/13 pT2, 6/6 pT3, 4/5 pT4. (**Table .2** & **Fig. 2**).

ERβ expression was positive in 19 (79%) (6 strong, 7 moderate, 6 weak) out of 24 MI and 10 (38.5%) (2 strong, 5 moderate, 3 weak) out of 26 NMI tumors. Higher ERβ expression was associated with muscle invasive compared to that of non-muscle invasive urothelial carcinoma (p-value 0.004), (**Table .2** & **Fig. 1,2**).

ERβ expression is significantly associated with the presence of lymphovascular emboli (p value 0.045). However, in cystectomy specimen with lymph node metastasis 7 out of 8 (87.5%) cases were ERβ positive but it wasn't statistical significance (p value 0.825). There was no statistical significance between ERβ expression and the presence of perineural invasion (p value 0.219) or the presence of bilharzial ova (p value 0.647).

Table 2. Relation between ERβ expression and clinicopathological characteristics:

Variables	ERβ negative (21)	ERβ positive (29)	P value
Age			
Mean	62.04	58.5	0.16
SD	± 8.7	± 9.8	
Gender			
Male (34)	12 (35%)	22 (65%)	0.161
Female (16)	9 (56%)	7 (44%)	
Tumor grade			
Low (22)	14 (64%)	8 (36%)	0.006*
High (28)	7 (25%)	21 (75%)	
Tumor stage			
Ta (20)	13 (65%)	7 (35%)	0.032*
T1 (6)	3 (50%)	3 (50%)	
T2 (13)	4 (31%)	9 (69%)	
T3 (6)	0 (0%)	6 (100%)	
T4 (5)	1 (20%)	4 (80%)	
Muscle Invasion			
NMI (26)	16 (62%)	10 (38%)	0.004*
MI (24)	5 (21%)	19 (79%)	
Tumor pattern			
Papillary (27)	16 (59%)	11 (41%)	0.007*
Nonpapillary (23)	5 (22%)	18 (78%)	
LN involvement			
Without (6)	1 (17%)	5 (83%)	0.825
With (8)	1 (12.5%)	7 (87.5%)	
LV Invasion			
absence (45)	21 (47%)	24 (53%)	0.045*
presence (5)	0 (0%)	5 (100%)	
PN Invasion			
Absence (48)	21 (44%)	27 (56%)	0.219
Presence (2)	0 (0%)	2 (100%)	
Bilharziasis			
Absence (44)	19 (43%)	25 (57%)	0.647
presence (6)	2 (33%)	4 (67%)	

Chi square test were used, significant*

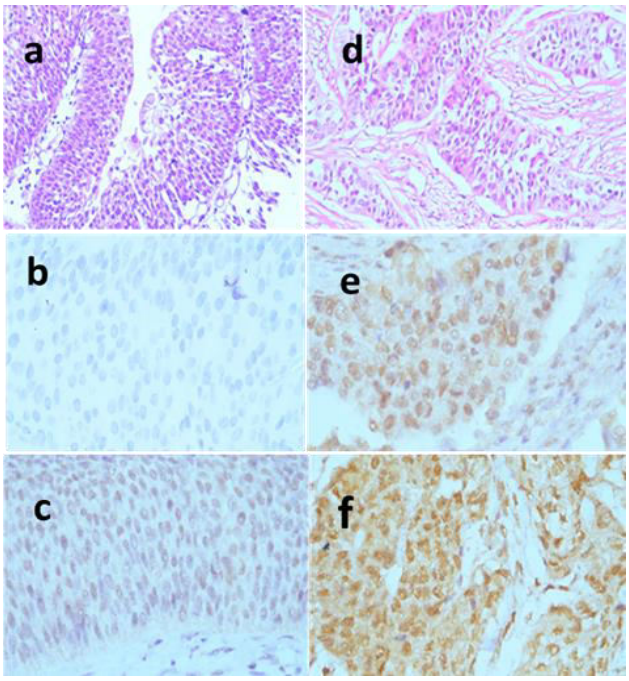

Fig.1: a) Low grade, noninvasive UC (H&E) x200. **b)** ERβ expression in non-invasive low-grade UC, negative expressionx400. **c)** ERβ expression in non-invasive low-grade UC, weak expressionx400. **d)** High grade, invasive UC (H&E) x200. e) ERβ expression in invasive high-grade UC, moderate expression x400. f) ERβ expression in invasive high-grade UC, strong expressionx400.

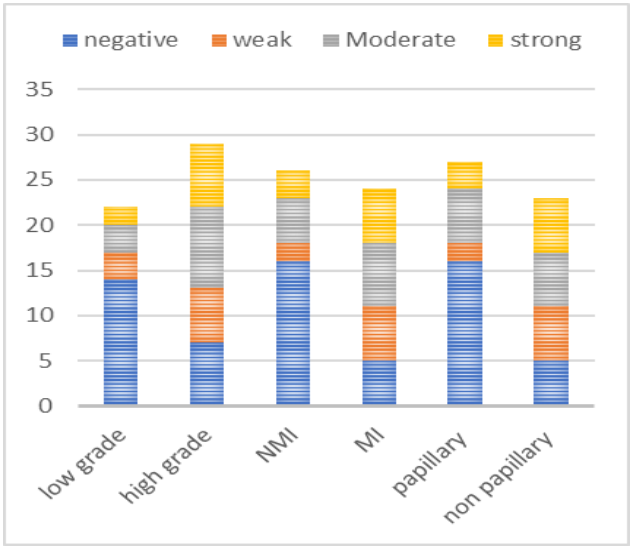

Fig.2: ERβ expression in the studied cases according to tumor grade, muscle invasion and tumor pattern.

Discussion

Urothelial carcinoma is the most frequent type of bladder cancer, which can be treated using a variety of techniques, from surveillance to radical cystectomy **(Magers et al., 2019).** Studies of estrogen receptors in bladder cancer tissues and cell lines have shown that ERβ is the most common subtype. The development of bladder cancer cells has been shown to be stimulated by increased ERβ activity and inhibited by antiestrogens in recent in vitro studies. In spite of this, the function of ER in bladder cancer in vivo is poorly known **(Han et al., 2012)**

In the current study, we examined 50 specimens of urothelial carcinoma: 29 (58%) (9 weak, 12 moderate and 8 strong) of the studied cases were ERβ positive similar to the results of most of the international research **Miyamoto et al. (2012)** 49%, **Malik et al. (2014)** 54%, **Al-Nandy and Alshenay, (2018)** 52.9%, **Nguyen et al. (2017)** 60%.

More than half of cases of non-papillary UC showed ERβ expression with significant difference compared to papillary tumors. **Croft et al. (2004)** also found that only 22% of the studied primary papillary UC were ERβ positive. This might be due to difference in sample size.

ERβ expression was present in most cases of high grade UC while it was expressed in only about one third of low grade UC (P value 0.006).These results are in agreement with **Miyamoto et al., (2012), Malik et al.,(2014)**, **Al-Nandy and Alshenay, (2018)** studies which demonstrated significant expression of ERβ in high grade UC. However, **Han et al. (2012)and Kontos et al. (2010)** found significant expression of ERβ in low grade UC. A possible explanation for this divergence is that the researchers in this study focused on NMI bladder cancer and utilized a more distinctive three-tier grading system. A meta-analysis found that ERβ expression is significantly greater in high-grade than low-grade UC, although there was no significant difference in ERβ expression between high- and low-grade NMIUC subgroups **Ide et al. (2017).**

According to the results of this research, ERβ expression is substantially linked to more advanced stages of UC (p value 0.03) and to MI vs NMI tumors (p value 0.004). **Shen et al. (2006)** also showed that ERβ expression was observed to be significantly different across Ta/T1 and T2/T3/T4 tumors, and to increase with tumor stage. **Nguyen et al. (2017)** investigated the expression of ERβ across different tumor stages, finding strong evidence that it is linked to muscle-invasive bladder cancer. **Al-Nandy and Alshenay, (2018)** stated the association between ERβ expression in high grade, MI and LVI.

In contrary to the previous results; **Kontos et al. (2010)** reported that low ERβ nuclear expression was associated with advanced tumour and they attributed these discordant results to the presence of distinct ERβ isoforms, difference in samples from different population and the difference in technical methodology.

In addition, the present work has shown that ERβ expression was significantly associated with the existence of LVI but not with nodal stage. This was also supported by **Al-Nandy and Alshenay (2018)**

To summarize, ERβ expression is associated with poor prognostic factors as high grade, advanced stages and the presence of lymphovascular emboli suggesting a role in tumorigenesis of UC.

Conclusion

The existence of lymphovascular invasion, high grade and more advanced stage are significantly associated with estrogen receptor beta expression in UC.

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