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Immunohistochemical Expression of Androgen and Estrogen Receptors and their Prognostic Significance in Urothelial Carcinoma of the Urinary Bladder

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Abstract

Background: Bladder cancer is the most common malignancy affecting the urinary tract. It is the fourth most common cancer in men and the ninth most common in women worldwide. It is at least three times more common in males than females suggesting the role of sex hormones in initiation and progression of bladder cancer. The purpose of this study is to detect the immunohistochemical expression of (AR) and (ER_β) in urothelial carcinoma and correlate their expression with the known prognostic parameters of urothelial carcinoma to illustrate their prognostic role.

Material and Methods: Seventy cases of urothelial carcinoma of the urinary bladder in the form of radical cystectomy (15 specimens) and Transurethral Resection of the Tumour (TURT) (55 specimens) were collected retrospectively. They were stained by H & E, AR and ER_β for immunohistochemical study. The relationship between their expression and the available clinicopathological features were evaluated.

Results: AR/ER_β was positive in (62.9%/52.9% respectively) of the studied cases. Significantly lower expression of AR/higher expression of ER_β were found in high-grade tumours (52.4%/66.7% respectively) (p-value=0.025, 0.005 respectively) and in muscle invasive tumours (48.4%/71% respectively) (p-value=0.026, 0.007 respectively).

Conclusions: AR and ER_β expression were significantly correlated with the tumour grade and degree of invasion suggesting the suitability of AR and ER_β as prognostic markers of urothelial carcinoma. High AR expression was associated with favorable prognosis of urothelial carcinoma in contrast to high ER_β expression which was associated with bad prognosis of urothelial carcinoma.

Key Words: *Androgen – Estrogen – Receptor – Bladder cancer – Urothelial – Immunohistochemistry – Prognosis.*

Introduction

BLADDER Cancer (BC) is the most common malignancy in the urinary tract and Urothelial Carcinoma (UC) is the predominant histological type [1]. Urothelial carcinoma is responsible for death of 165000 persons annually worldwide according to the International Agency for Research on Cancer and the World Health Organization, with the highest mortality rates among Egyptian males [1]. It is more common in middle and old age [2]. Females presented with the disease at older age than males [3]. Urothelial carcinoma is the 4th most common cancer in men and the 9th in women worldwide [1]. In Gharbia government, bladder cancer ranks the 3rd in both sexes being the 2nd in males and the 7th in females [4]. It is at least three times more common in men than women [1]. Excessive exposure to carcinogens, e.g. cigarette smoke and industrial chemicals, has been suggested to be a cause of higher incidence of bladder cancer in males [5]. Even, after controlling these carcinogens, men still have a higher risk than women [6].

In the light of the previous facts, existence of a relationship between sex hormones and cancer bladder is suggested. Data from animal and human studies suggested that sex hormones have important physiological effects on the lower urinary tract [7]. Sex steroids act by binding to their receptors in target cells including Androgen Receptors (ARs) and Estrogen Receptors (ERs) [8]. These receptors have been detected in normal bladder urothelium [9]. The AR and ER signaling pathways affect bladder cancer development and progression [8]. Bladder cancer management has remained essentially unchanged, with no new effective treatment options approved in the past few decades [10]. Treatment of non-muscle invasive bladder cancer involves transurethral resection of the tumour followed by intravesical chemo-or immunotherapy but muscle-invasive cancer requires radical cystectomy and systemic chemotherapy [11]. Many patients receiving BCG develop either local or systemic side

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effects [10]. The effects of sex hormones on bladder cancer cells need to be studied, which might help in development of prognostic biomarkers and new therapies.

Material and Methods

This study was carried out on 70 cases of urothelial carcinoma of the urinary bladder (56 males and 14 females, age ranged between 28 and 88 years). These cases were collected retrospectively from the archives of Pathology Department, Faculty of Medicine, Tanta University and from some private laboratories during the period of the research from 2015 to 2017 and patients' data were obtained from files of surgery and oncology reports. Approval from Research Ethics Committee (REC), Faculty of Medicine, Tanta University, was taken antecedent to conducting study. Tissue specimens were in the form of radical cystectomy (15 specimens) and Transurethral Resection of the Tumour (TURT) (55 specimens). After histopathological evaluation, tumors were graded according to the WHO 2016 of urothelial neoplasia, and they were classified as low grade and high grade urothelial carcinoma. Tumors were staged according to American Joint Committee (AJCC), TNM pathologic staging of urinary bladder. Immunohistochemical staining was performed on 10% formalin fixed, paraffin embedded tissue blocks for evaluation of AR and ERP expression. Sections were immunohistochemically labeled, using primary antibodies to AR (EPR3778 clone, rabbit monoclonal antibody, 0.1ml, dilution 1:50; Dako, Egypt) and ER P (AR441 clone, mouse monoclonal antibody, 0.1]ml, dilution 1:300; Abcam, Egypt). AR and ER P staining was detected as brownish nuclear staining and all these stains were manually scored by the German Immunoreactive Score based on multiplying percentage of immunoreactive cells {0%=(0), 1-10%=(+1), 11-50%=(+2), 51-80%=(+3), 81-100%=(+4)} by staining intensity {Negative (+0), weak (+1), moderate (+2), strong (+3)}. Scores (range 0-12) were considered negative (0; 0-1), weakly positive (+1; 2-4), moderately positive (+2; 6- 8) and strongly positive (+3; 9-12) [8]. Chi-square test and Spearman's correlation coefficient test were used as tests of significance to evaluate the association between categorized variables and p-value <0.05 was considered statistically significant.

Results

Different clinicopathological criteria of 70 bladder specimens were evaluated (Table 1), then correlated with AR and ER P expression (Table 2).

Table (1): Clinicopathological characteristics of the studied cases.

Clinicopathological features	No.	%
1- *Age:*		
<50	13	18.6
50	57	81.4
2- *Gender:*		
Male	56	80
Female	14	20
3- *Size:*		
<3cm	55	78. 6
3cm	15	21.4
4- *Multiplicity:*		
Single	36	51.4
Multiple	34	48. 6
5- *Grade:*		
Low	28	40
High	42	60
6- *Degree of muscle invasion:*		
NMI (Ta,T1)	39	55.7
MI (T2, T3, T4)	31	44.3
7- *Level of in* vasio*n at cystectomy specimens:*	15	
pT2a	1	6.7
pT2b	6	40
pT3a	2	13.3
pT3b	1	6.7
pT4a	4	26. 6
pT4b	1	6. 7
8- *L.N metastasis at cystectomy specimens:*	15	
Without	11	73.3
With	4	26.7
9- *Concomitant CIS:*		
Without	68	97.1
With	2	2.9
10- *Perineural invasion:*		
Without	61	12.9
With	9	87.1
11- *Lympho-vascular invasion:*		
Without	62	88.6
With	8	11.4
12- *Recurrence after treatment:*		
Without	67	95.7
With	3	4.3

CIS : Carcinoma in Situ.
NMI : Non-Muscle Invasive.
MI : Muscle Invasive.

Table (2): AR and ERβ expression correlated with clinicopathological characteristics.

Variables	AR			ER_{β}		
	–ve (n=26)	+ve (n=44)	*p*-value	–ve (n=33)	+ve (n=37)	*p*-value
1- *Age:*						
<50 (13)	6 (46.2%)	7 (53.8%)	0.456	6 (46.1%)	7 (53.9%)	0.529
50 (57)	20 (35.1%)	37 (64.9%)		31 (54.4%)	26 (45.6%)	
2- *Gender:*						
Male (56)	20 (35.7%)	36 (64.3%)	0.621	27 (48.2%)	29 (51.8%)	0.119
Female (14)	6 (42.9%)	8 (57.1%)		10 (71.4%)	4 (28.6%)	
3- *Size:*						
<3cm (55)	17 (30.9%)	38 (69.1%)	0.039*	30 (54.6%)	25 (45.4%)	0.018*
≥3cm (15)	9 (60%)	6 (40%)		3 (20%)	12 (80%)	
4- *Multiplicity:*						
Single (36)	12 (33.3%)	24 (66.7%)	0.497	22 (61.1%)	14 (38.9%)	0.016*
Multiple (34)	14 (41.2%)	20 (58.8%)		11 (32.4%)	23 (67.6%)	
5- *Grade:*						
Low (28)	6 (21.4%)	22 (68.6%)	0.025*	19 (67.9%)	14 (32.1%)	0.005*
High (42)	20 (47.6%)	22 (52.4%)		9 (33.3%)	28 (66.7%)	
6- *Degree of invasion:*						
NMI (39)	10 (25.6%)	29 (74.4%)	0.026*	24 (61.5%)	15 (38.5%)	0.007*
MI (31)	16 (51.6%)	15 (48.4%)		9 (29%)	22 (71%)	
7- *Invasion level (15):*						
T2 (7)	4 (57.1%)	3 (42.9%)	0.047*	3 (42.9%)	4 (57.1%)	0.042*
T3 (3)	2 (66.6%)	1 (33.3%)		1 (33.3%)	2 (66.7%)	
T4 (5)	4 (80%)	1 (20%)		0 (0%)	5 (100%)	
8- *L.N metastasis (15):*						
Without (11)	7 (63.6%)	4 (36.4%)	0.679	3 (27.3%)	8 (72.7%)	0.929
With (4)	3 (75%)	1 (25%)		1 (25%)	3 (75%)	
9- *Concomitant CIS:*						
Without (68)	25 (36.8%)	43 (63.2%)	0.703	33 (48.5%)	35 (51.5%)	0.175
With (2)	1 (50%)	1 (50%)		0 (0%)	2 (100%)	
10- *Perineural invasion:*						
Without (61)	20 (32.8%)	41 (67.2%)	0.049*	31 (50.8%)	30 (49.2%)	0.033*
With (9)	6 (66.7%)	3 (33.3%)		2 (22.2%)	7 (77.8%)	
11- *L-V invasion:*						
Without (62)	21 (33.9%)	41 (66.1%)	0.115	31 (50%)	31 (50%)	0.041*
With (8)	5 (62.5%)	3 (37.5%)		2 (25%)	6 (75%)	
12- *Recurrenc:*						
Without (67)	24 (35.8%)	43 (64.2%)	0.278	33 (49.3%)	34 (50.7%)	0.279
With (3)	2 (66.7%)	1 (33.3%)		0 (0%)	3 (100%)	

AR expression was detected in 44 cases (62.9%) (12 weak, 18 moderate, 14 strong). AR expression was not significantly correlated with the age and gender (*p*-value=0.456, 0.621 respectively). There was a significant correlation between AR expression and tumour size (*p*-value=0.039).

AR expression was not significantly correlated with the number of urinary bladder masses (*p*-value=0.497). AR expression was significantly correlated to tumour grade (*p*-value=0.026). AR expression was higher in low grade cases (78.5%) Fig. (1A) than high-grade cases (52.4%) Fig. (1B).

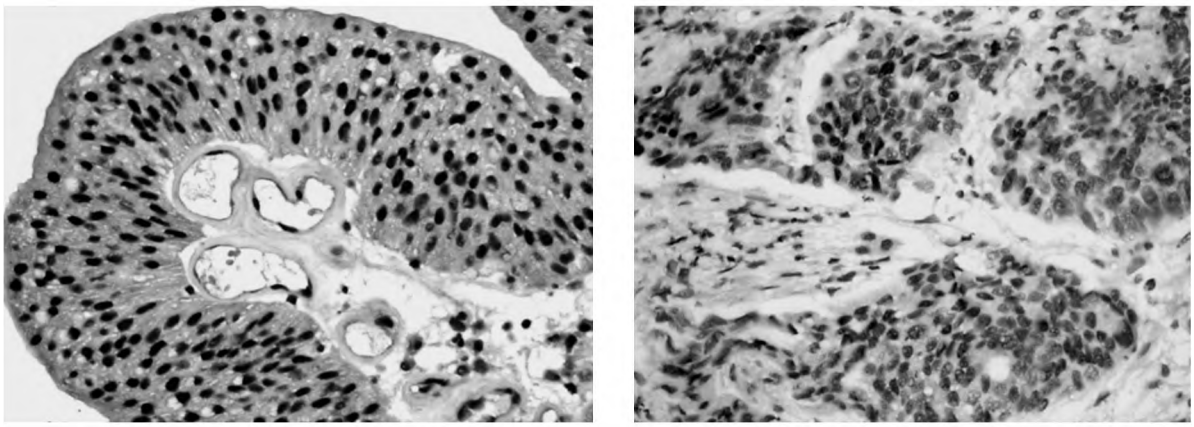
AR expression was significantly correlated with degree of muscle invasion (*p*-value=0.026) with higher AR expression in NMI tumours (pTa, pT1) (74.4%) opposite to only 48.4% of MI (pT2, pT3, pT4). In cystectomy specimens, there was a significant association between AR expression and the level of invasion (*p*-value=0.046) with negative correlation coefficient (r_s) denoting loss of AR expression in more invasive tumours. AR expression wasn't significantly correlated with lymph node metastasis in cystectomy specimens and lesions with concomitant CIS (*p*-value=0.679, 0.703 respectively). There was a significant corre-

lation between AR expression and perineural invasion (p-value=0.049). AR expression wasn't significantly correlated with lympho-vascular invasion (p-value=0.115). In the three cases that showed recurrence after treatment with TUR followed by intravesical BCG instillation, two primary cases (66.7%) were AR negative and one case (33.3%) was AR+2.

ER_P expression was positive in 37 cases (52.9%) (11 weak, 20 moderate, 6 strong). ER_P expression wasn't significantly correlated with the age and gender (p-value=0.592, 0.119 respectively). ER_P expression was significantly correlated with tumor size and multiplicity (p-value=0.018, 0.016 respectively). ER_P expression was significantly correlated to the tumour grade in the studied cases (p-value=0.005). Higher positive ER_P in high-grade cases (66.7%) than low-grade cases (32.1%) Fig. (2A,B).

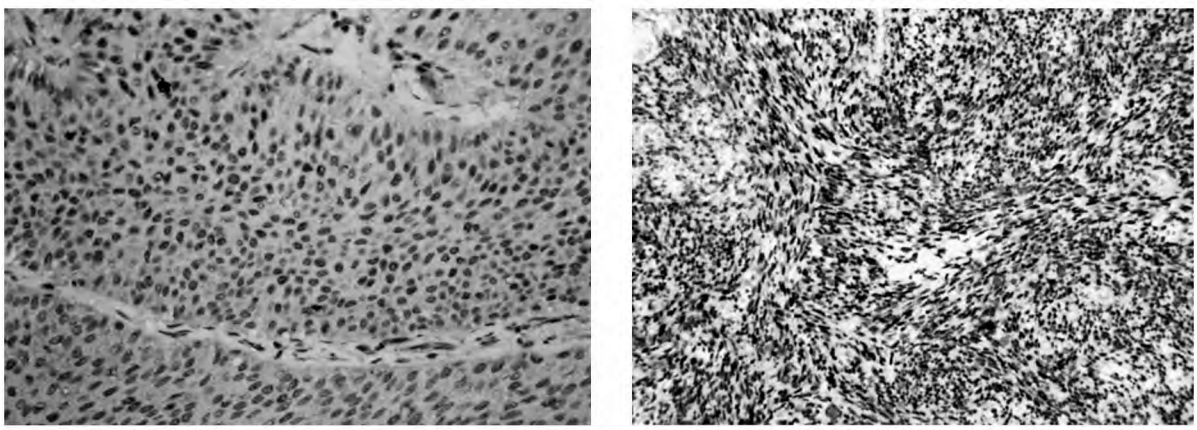
ER_P expression was significantly correlated with degree of muscle invasion (p-value=0.007) with lower ER_P expression in NMI tumours (38.5%) opposite to 71% of MI Fig. (3A). In cystectomy specimens, there was a significant association between ER_P expression and invasion level (p-value=0.033) with positive correlation coefficient (r_s) denoting high ER_P expression in more invasive tumours. In cystectomy specimens with lymph node metastasis, three cases (75%) showed positive expression, but it did not reach the statistically significant level (p-value=0.929) Fig. (3B).

Lesions with concomitant CIS showed no significant correlation with ER_P expression (p-value= 0.175). ER_P expression was significantly correlated with perineural and lympho-vascular invasion (p-value=0.033, 0.041 respectively). The three cases (100%) that showed recurrence after treatment were ER_P positive, but it did not reach the statistically significant level (p-value=0.279).



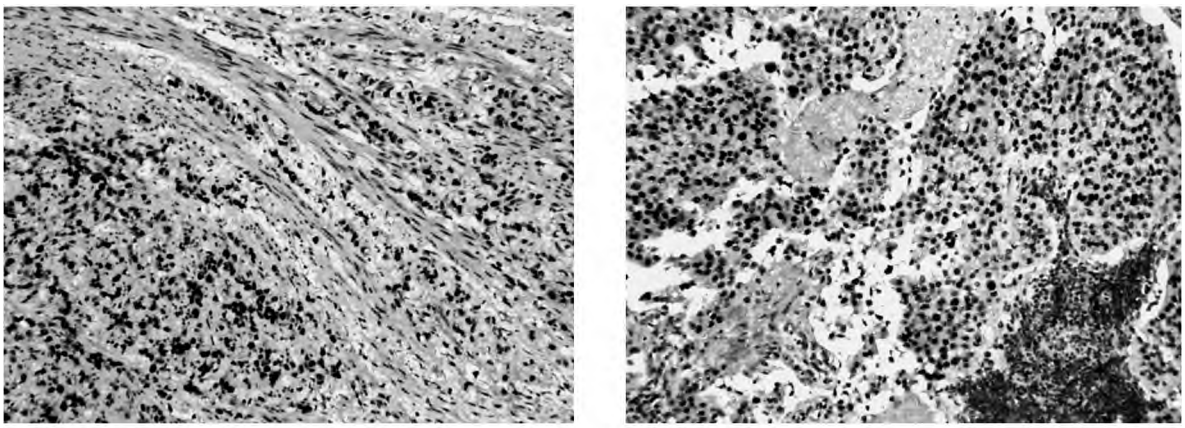
(A) (B)

Fig. (1): (A) Low grade non-invasive papillary UC showing strong positive AR immunoreactivity (+3). (X400). (B) High grade UC infiltrating lamina propria with weak positive AR immunoreactivity (+1). (X400).



(A) (B)

Fig. (2): (A) Low grade non-invasive UC showing weak positive ER_P immunoreactivity (+1). (X200). (B) High grade UC with sarcomatoid differentiation showing strong positive ER_P (+3). (X200).



(A) (B)

Fig. (3): (A) High grade UC infiltrating muscularis propria showing strong positive ER β (+3). (X200). (B) Lymph node metastasis of UC showing strong positive ER β (+3). (X200).

Discussion

AR and ERβ are members of nuclear receptor superfamily [3]. Androgen/androgen receptor signaling pathway up-regulates the expression of active β-catenin also affects p53 tumor suppressor functions [12]. A retrospective study involving patients who received androgen deprivation therapy for prostate cancer showed a significantly lower incidence of subsequent BC [13]. Higher AR expression associated with low grade tumour, non-muscle invasive tumour, size less than 3cm and absent perineural invasion. Loss of AR expression was reported in cases with lymph node metastasis and risk of recurrence, but it did not reach the statistically significant level. From that, higher AR expression was associated with better prognosis of urothelial carcinoma which was in agreement with some studies [8,14,15]. In contrast to a study reported that AR expression had no prognostic value (124) and another one reported bad prognosis in AR-positive patients with higher rate of invasion, metastasis and recurrence [17]. High AR expression was significantly correlated with low-grade cases (p-value=0.026) which was in agreement with Miyamoto, et al., (p-value=0.023) [8]. In contrast, Mashhadi, et al., reported a significant association between AR expression and high-grade tumors (p-value=0.024) [17]. High AR expression was significantly correlated with NMI cases (p-value=0.026) which was in agreement with Miyamoto, et al. (2012) (p-value=0.018) [8]. In contrast to Mir, et al. (2011) who concluded loss of AR expression in NMI tumours (p-value= 0.048) [16].

ERβ is the dominant estrogen receptors expressed in urothelium [10]. Estrogen affects cell cycle progression through up-regulation of both cyclin D1 and cyclin E [18].

Higher ERβ expression in the current study was significantly correlated with high grade tumours, more invasive tumours, size more than 3cm, multiple lesions in the urinary bladder, tumours with perineural and lympho-vascular invasion. High ERβ expression was detected in cases with lymph node metastasis and risk of recurrence, but it did not reach the statistically significant level. From that, high ERβ expression was associated with bad prognosis of urothelial carcinoma which was in agreement with most studies [8,19]. In contrast Bangmin, et al., [20] reported favorable prognosis in ERβ positive patients. High ERβ expression was significantly correlated with high-grade tumours (p-value=0.005) which was in agreement with Miyamoto, et al. (2012) (p-value <0.001) [8]. In contrast, Bangmin, et al., reported a significant association between ERβ expression and low-grade tumors (p-value=0.037) [20]. High ERβ expression was significantly correlated with MI cases (p-value=0.007) which was in agreement with Miyamoto, et al. (2012) (p-value <0.001) [8]. In contrast to Kontos, et al., who reported higher positive ER expression in NMI tumours (p-value=0.001) with good prognosis [21].

Conflict of interest:

None declared.

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التعبير الهيستوكيميائى المناعى لمستقبلات الإندروجين والإستروجين وعلاقتها بتطور المرض فى سرطان الغشاء الطلائى للمثانة البولية

سرطان الغشاء الطلائى للمثانة البولية هو رابع أكثر أنواع السرطانات شيوعا لدى الرجال والتاسع لدى النساء فى جميع أنحاء العالم ويعتبر ثلاث مرات أكثر شيوعا فى الذكور من الإناث. ويعتقد أن الإفراط فى التعرض للمواد المسرطنة سبب إرتفاع معدلات الإصابة بسرطان المثانة لدى الرجال. ومع ذلك، وبعد السيطرة على هذه العوامل المسببة للسرطان، لا يزال لدى الرجال خطر أعلى بكثير من النساء ولذلك إقترح وجود علاقة بين سرطان المثانة والهرمونات الجنسية والتى تعمل عن طريق مستقبلات مثل الإندروجين ومستقبلات الإستروجين فى الخلايا المستهدفة. تهدف هذه الدراسة إلى دراسة التعبير عن مستقبلات الإندروجين والإستروجين فى الغشاء الطلائى للمثانة البولية وعلاقتها بالعلامات المنذرة المعروفة عن هذا السرطان لتوضيح دورها النذير. وقد ظهرت مستقبلات الإندروجين كصبغة بنية لنوايا خلايا الغشاء الطلائى فى ٦٢.٦٪ من الحالات. ووجد أن زيادة التعبير عن هذه المستقبلات له قيمة إحصائية فى الأورام ذات الدرجة الأقل والتى لم تصل إلى عضلات المثانة والأورام فى المراحل الأقل تقدما، الأورام ذات الحجم أقل من ٣سم والتى يغيب فيها إختراق الأعصاب. أما مستقبلات الإستروجين بيتا فقد ظهرت أيضا كصبغة بنية لنوايا خلايا الغشاء الطلائى للمثانة البولية ولكن فى ٥٢.٩٪ فقط من الحالات. وكان زيادة التعبير عنها له قيمة إحصائية فى الأورام ذات الدرجة الأعلى والتى تصل إلى عضلات المثانة والأورام فى المراحل الأكثر تقدما، الأورام ذات الحجم أكثر من ٣سم والمتعددة الأماكن والتى يوجد بها إختراق الأعصاب والأوعية الليمفاوية والدموية. زيادة ظهور مستقبلات الإندروجين من العلامات الإيجابية أما زيادة الإستروجين بيتا فإنه من العلامات المنذرة السيئة.