

Original Research Article

The Role of Estrogen Receptors Immunohistochemical Study in Prognosis of Bladder Transitional Cell Carcinoma

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Abstract

Urothelial Bladder cancer (UBC) is a common and complex disease that have various internal and external risk factors and represent 90% of bladder cancer. Various studies involving immunohistochemical staining of estrogen receptors (ER) have rendered inconsistent results regarding the expression of ER α and ER β for different bladder cancer stages and grades. Ninety five Paraffin in embedded blocks of UBC (68 male and 27 female) were studied by immunohistochemical technique for detection of estrogen receptor expression and its correlation to some of prognostic parameters such as sex, age, grade and stage of UBC. The study was carried out in the Department of Pathology\ College of Medicine\ Babylon University during the period from November 2013 to August 2015. Mean age of all cases was 64.6 and they ranged from 30 to 90 years old. The results showed that there were low rate of estrogen receptor expression (only 15 positive cases) and no significant correlation between estrogen receptor expression and the involved prognostic parameters (p value > 0.05). So estrogen receptor expression cannot be used to assess the prognostic outcome for Urothelial bladder cancer.

Key words: Estrogen receptor, Urothelial bladder cancer , Immunohistochemical technique , Expression.

دور مستقبلات الاستروجين في دراسة التعبير المناعي النسيجي في تشخيص والتنبؤ بسرطان الخلايا الانتقالية للمثانة

الخلاصة

سرطان الخلايا الانتقالية للمثانة هو مرض شائع ومعقد وله مختلف عوامل الخطورة الداخلية والخارجية وانها تمثل ٩٠% من سرطانات المثانة. بينت الدراسات المختلفة التي تنطوي على التقنية المناعية (التعبير المناعي النسيجي). لمستقبلات هرمون الاستروجين على نتائج متضاربة بشأن التعبير عن مستقبلات هرمون الاستروجين الفا وبيتا لمختلف مراحل الإصابة بسرطان المثانة وكذلك درجة الإصابة. أجريت هذه الدراسة على ٩٥ عينة من مرضى سرطان الخلايا الانتقالية للمثانة (٦٨ حالة من الذكور و ٢٧ حالة من الإناث) للكشف عن العلاقة بين تعبير مستقبلات هرمون الاستروجين وبعض العوامل التكهنية للمرض مثل الجنس والعمر ودرجة الإصابة ومرحلة السرطان. تمت الدراسة في جامعة بابل / كلية الطب / فرع الامراض والطب العدلي للفترة بين شهر تشرين الثاني من سنة ٢٠١٣ لشهر اب من سنة ٢٠١٥ وكان متوسط عمر جميع الحالات ٦٤,٦ وأنها تراوحت بين ٣٠ الى ٩٠ سنة. ولقد أظهرت النتائج أن هناك انخفاض في معدل تعبير مستقبلات هرمون الاستروجين (١٥ حالة موجبة) وعدم وجود ارتباط او علاقة بين تعبير مستقبلات هرمون الاستروجين والعوامل التكهنية المعنية بالدراسة (p<٠,٠٠٥). لذلك فان التعبير لمستقبلات هرمون الاستروجين لا يمكن استخدامها لتقييم والتكهن لنتائج سرطان الخلايا الانتقالية للمثانة.

الكلمات المفتاحية: مستقبلات هرمون الاستروجين ، سرطان الخلايا الانتقالية للمثانة ، التعبير المناعي النسيجي ، التعبير النسيجي.

Introduction

Bladder cancer (BC) is the commonest malignant tumor in the urinary system, which represents the ninth most common malignant tumor in the world with male predominance, and an estimated male-female ratio of 4:1 [1]. Bladder cancer represent one of the 10 most common malignant tumor in the world [2].

In Iraq, bladder cancer comprise the fourth most common malignant tumor in male and eighth most common cancer in female [3]. The bladder cancer represent fourth most common cancer in the United States, and there was about 72,570 new cases diagnosed and 15,210 deaths in the US only in 2013 [4]. It represents the most common tumor in Europe, and in incidence and mortality rates represents the ninth tumor in Europe [5]. Bladder cancer can occur in young persons, and more than 90% of new cases diagnosed in persons higher 55 years of age [6].

Malignant bladder tumor can arise because of multiple complex external and internal factors, including smoking, age, gender, genetic, exposure to chemical carcinogens [7]. Urothelial carcinoma of the bladder (UCB) is the most common histological subtype of bladder cancer and represent more than 90% and the remaining 10% are squamous cell carcinomas (3% to 8%), adenocarcinomas (1%) and other types [8]. Hormonal factor play an important role in the development of bladder cancer and this based on the male predominance of UBC independent of occupational exposure and tobacco smoking [9].

Estrogen receptor (ER) are members of the superfamily of nuclear hormone receptors play an important role in almost all phases of physiology and pathology, including reproduction, metabolism, immune function, maintenance of bone density, and growth of estrogen-responsive tumors, including breast and endometrial cancers, and they act by transduce extracellular signals into transcriptional response [10]. The ER presents in two main forms, ER α

encoded by a gene on chromosome 6; and ER β encoded by a gene on chromosome 14, which expressed in different tissues [11].

The estrogen receptor genes, ER α and ER β , expression and patterns of functions are widely different in tissue [12]. The ER β protein represent the main ER in bladder, and have expression of about 77% of UCB, while the ER α protein expression is infrequent (<5%) [13, 14, 15]. In some studies, they showed that the ER α expression is located more in the posterior region of the bladder neck and in smooth muscle of the trigone [16, 17].

A recent study shows that, there was no relationship between the ER α and tumor progression, recurrence, and cancer specific survival. The expression of ER α was not associated with the prognosis of bladder cancer as it appears in breast [18].

Patients and Methods

This study included 95 Paraffin embedded blocks for cases of transitional cell carcinoma of urinary bladder that have been collected from surgical specimens after surgical resection, TUR, or biopsy of the tumors. From these 95 cases, 87 were collected from Karbala city (laboratory of Histopathology of Imam AL Hussein Medical City and from AL-Sajjad private histopathological laboratory in Karbala), and 9 cases from laboratory of Histopathology of AL Hilla Teaching Hospital in Babylon. The cases included 68 male (71.58%), and 27 female (28.42%) with primary transitional cell carcinoma of urinary bladder. At first, the cases re-examined by haematoxylin and eosin stain for histopathological study to confirm the primary diagnosis of the cases, stage and grade of the tumor.

In the next step, all the slides stained with immunohistochemical stain specific for Estrogen receptor. Reading the result done by Allred scoring system, which based on examination of all stained tumor cells (nuclear stain) on the slide. Allred scoring system is the well-known scoring system for evaluate the nuclear expression of

Estrogen receptors in the tumor cells. It involves two parts:

1. Proportional score (PS). It represents the proportion of tumor cells with positive nuclear staining in power field under 40X and consists of five grades as following:

0 = no staining cells

1 = >0 to 1/100 of cells take the stain

2 = >1/100 to 1/10 of cells take the stain

3 = > 1/10 to 1/3 of cells take the stain

4 = > 1/3 to 2/3 of cells take the stain

5 = > 2/3 to 1 of cells take the stain

Results

From 95 cases, 15 cases were positive for estrogen receptor with frequency of (15.8%). From these 15 positive cases of estrogen receptor, 10 cases male (5 < 65 years old and five ≥ 65 years old) and five cases female (1 < 65 years old and four ≥ 65 years old). Positive low grade were 11 cases (5 < 65 years old and 6 ≥ 65 years old) and positive high grade were four cases (1 < 65 years old and 3 ≥ 65 years old). Positive stage 0a were seven cases (5 < 65 years old and 2 ≥ 65 years old) [Ta (Non-invasive papillary carcinoma). N0 (No regional lymph node metastasis). M0 (No distant metastasis)]. Positive stage II [T2a, b (T2a means tumor invades superficial muscle, T2b means tumor

2. Intensity Score (IS). It represent the average staining intensity of all positive tumor cells and consists of three grades:

0 = negative (no stain)

1 = weak stain

2 = intermediate stain

3 = strong stain

A Total Score (TS) is the sum of PS plus IS (ranging from 0, 2–8). A positive result for estrogen receptor is defined as $TS \geq 3$, which was validated in numerous large clinical studies.

invades deep muscle). N0 (No regional lymph node metastasis). M0 (No distant metastasis) were eight cases (1 < 65 years old and 7 ≥ 65 years old). Mean age of all positive cases, positive male cases and positive female cases were 64.3, 62.3, and 68.2 respectively. There was no significant association between estrogen receptor expression and the involved parameters in the study which including age, sex, stage and grade of UBC (p value > 0.05). The distribution of patients with urothelial bladder Carcinoma by estrogen receptor expression were showed in the figure number one. Figures two and three show positive estrogen receptor expression.

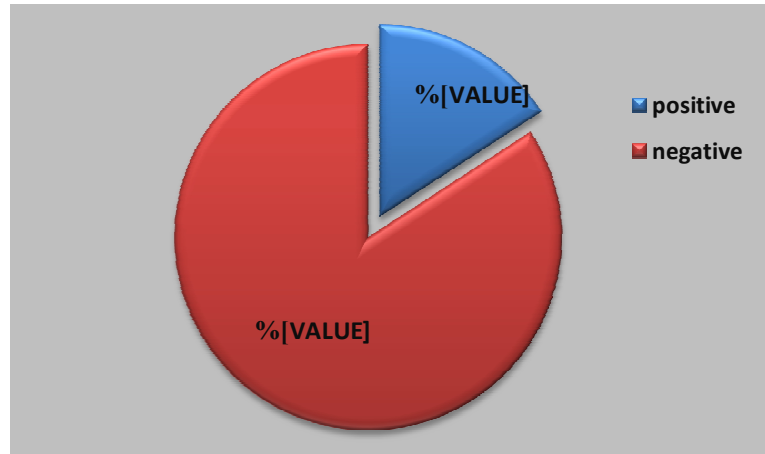


Figure 1: Distribution of patients with urothelial bladder Carcinoma by estrogen receptor expression

Table 1: The relation of estrogen receptor expression with age, sex, grade, and stage of tumor

Variables		Frequency (%)	Estrogen receptor expression		χ^2	P value
			Positive (15 cases)	Negative (80 cases)		
Age	< 65	39 (41.1%)	6	33	0.08	0.92
	≥ 65	56 (58.4%)	9	47		
Gender	Male	68 (71.6%)	10	58	0.211	0.646
	Female	27 (28.4%)	5	22		
Tumor Grade	Low grade	61 (64.2%)	11	50	0.645	0.422
	High grade	34 (35.8%)	4	30		
Tumor Stage	Stage 0a	54 (56.8%)	7	47	0.752	0.386
	Stage II	41 (43.2%)	8	33		

P value < 0.05

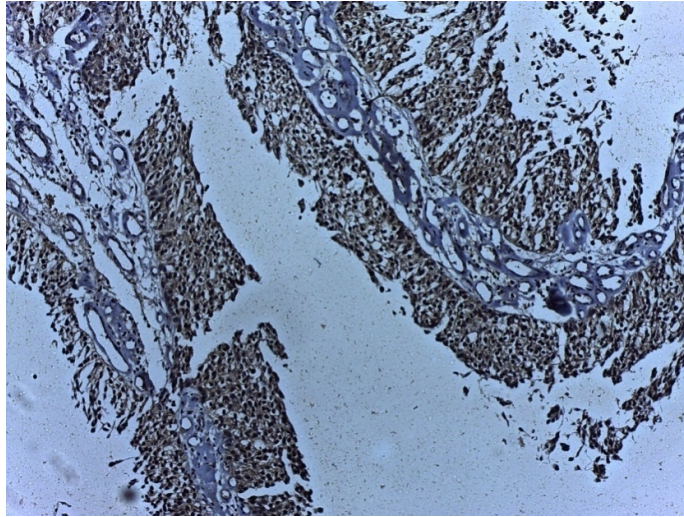


Figure 2: Urothelial bladder cancer with positive estrogen receptor expression (Immunohistochemical stain, x10), Allred score include: Intensity score = 3, Proportional score = 4, Total score = 7

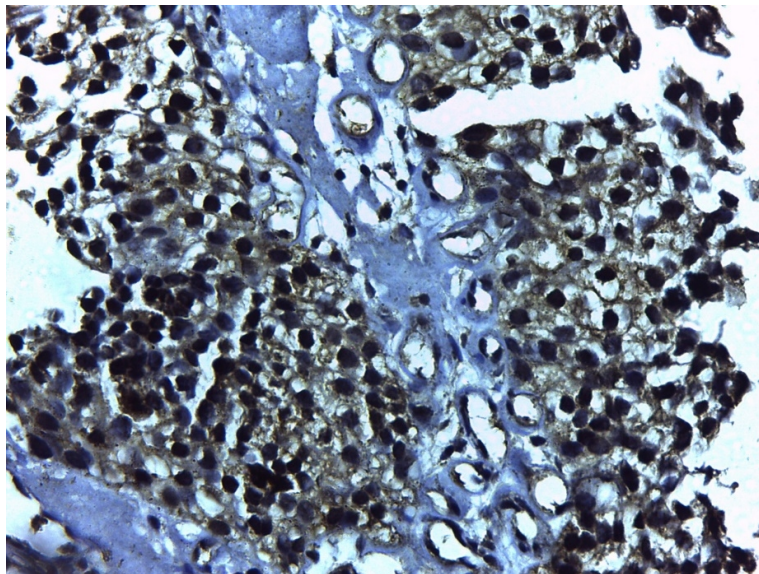


Figure 3: Urothelial bladder cancer with positive Estrogen receptor expression (Immunohistochemical stain, x40), Allred score include: Intensity score = 3, Proportional score = 4, Total score = 7

Discussion

This study was carried out for detection and assessment the expression of estrogen receptors in urothelial bladder cancer in Iraqi patients for male and female (Karbala city and Babylon city). There are many researches in the past and continuous until now trying to detect the role of sex hormone receptors in the development and progression of urothelial bladder cancer. In addition, study the relationship of receptor expression with the histopathological

characteristics of the tumor and its prognostic significance.

In the current study, there were only 15 cases from 95 cases (10 cases male and 5 cases female) showed positive estrogen receptor expression which represent 15.8% and this goes with other studies have been made to detect and study the presence of estrogen receptor in the lower urinary tract especially urinary bladder in both male and female.

In 1981, Saez and Martin , they did a research on the urinary bladder and the presence of estrogen receptor. They found that, there was a small percent of estrogen receptor in the posterior bladder, trigone, and in women present in proximal urethra, and this finding goes with the result of the current study[19]. Laor and colleagues in their research observed that there was a decrease in the recurrence rate of UBC in male patients with prostatic carcinoma treated by hormonal drugs and this agrees with the presence of estrogen receptor in male urinary bladder [20]. In 1998, Kaufmann with his coworkers made a study on detection of estrogen receptor in Urothelial bladder cancer. They take 185-tissue sample of Urothelial bladder cancer and found that there were low percentage of estrogen receptor alpha expression in Urothelial bladder cancer (18%), as in current study only 15.8% of 95 cases had positive estrogen receptor expression[21]. In addition, in 2001, there was a case report by Dellagramaaticas showed that male patient with cutaneous deposits of metastatic urothelial bladder cancer disappeared after 3 months of daily treatment with 10 mg of tamoxifen for gynecomastia, this result can show the effect of tamoxifen on estrogen receptor in male urothelial bladder cancer[22]. **Bolenz** and coworkers studied estrogen and progesterone receptors expression in urothelial carcinoma of the bladder. They made a study on 198 specimens of urothelial bladder cancer (156 male and 42 female), they showed that only 9 cases were positive for ER and divided in to 7 cases male and 2 cases female, and this result goes with the finding of the present study[23]. Arshad Rahmani and other workers studied IHC expression of estrogen and progesterone receptors in 125 cases of urinary bladder carcinoma. The Expression of ER receptor was noticed only in 18.4% of cases, and this finding agrees with the finding of present study[24].

Association of Estrogen receptor with age can be explain by many researches like that McGrath and his workers in their

research on bladder cancer and hormonal factor showed that the risk of urothelial bladder cancer more in postmenopausal women than premenopausal women [25]. Davis-Daoshowed that UBC more in old age and postmenopausal women[26].

Association of Estrogen receptor with the grade and stage of tumor can be explain by many researches like that Shan and workers studied ER and urothelial bladder cancer on 38 specimens. They noticed that the ER positivity rates were 42% for low grade while 13% for high grade [27]. **Hiroshi et al.** they studied the expression of estrogen receptor and its significance in UBC. They act on 188 specimens of UBC. They showed that ER expression reduced in high grade in comparison with low grade UBC [28]. Croft and coworkers assessed the presence of estrogen receptor in 92 specimens of UBC. He found 11% positive cases and, the invasive UBC were more to be in ER positive cases [29]. In addition, there were other researches showed that estrogen receptor has been implicated in more advanced stages of UBC and worse bladder survival among females[30, 31].

Conclusion

Few cases of urothelial bladder cancer have express estrogen receptor (only 15 cases) (15.8%). Mean age of female positive cases were higher than mean age of male positive cases (68.2 > 62.3). Estrogen receptor expression was more in old aged female and early menopause. Low rate of estrogen receptor alpha expression in UBC that mimic other studies in other countries for the same object. There were no significant association between estrogen receptor expression and tumor grade, stage, sex, and age. (P value > 0.05). So estrogen receptor expression cannot be used to assess the prognostic outcome for urothelial bladder cancer.

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