Expression of Estrogen receptor beta in Squamous cell carcinoma of bladder cancer

Hassan R[1], Zaky A[2], Elsaba T[2], Sayed MA[3], Zeidan A[1]

[1] Department of Medical Oncology and Hematology Department, South Egypt Cancer Institute, Assiut University, Assiut, Egypt.
[2] Department of Pathology, South Egypt Cancer Institute, Assiut University, Assiut, Egypt.
[3] Department of Urology, Assiut University, Assiut, Egypt.

Correspondence should be addressed to
Email: medicine3005@yahoo.com
Tel: + (20) 01016809167

Abstract

Background: bladder cancer is a major oncological problem; it has a high incidence rate with high mortality. Two pathological types are detected in bladder cancer: urothelial and non-urothelial. In Egypt; it was the first common malignancy in males but now it is the second after hepatocellular carcinoma, with the highest mortality worldwide. The two main histopathological types in Egypt are transitional cell carcinoma and squamous cell carcinoma. Treatment of bladder cancer has limited options and poor response. 5-year survival in metastatic bladder cancer is 5%. Studies are ongoing trying to find a new drug that can improve disease outcomes. Our study evaluates the expression of Estrogen receptor Beta in bladder cancer and study the association with the clinicopatological factors.

Key words: Estrogen receptor beta, bladder cancer, squamous cell carcinoma, clinicopathological factors.

Introduction:

Bladder cancer (BC) is the ninth most common cancer worldwide [5]. There are two histopathological types of bladder cancer (urothelial and non-urothelial). Urothelial bladder cancer accounts for about 95% of all types [6].The remaining bladder cancer types are non-urothelial including: squamous cell carcinoma, adenocarcinoma, small cell carcinoma, sarcomas, lymphoma, malignant melanoma, and undifferentiated carcinoma.

In Egypt, bladder cancer is the second most common malignancy among men, 30% of the cases are Squamous cell carcinoma type [18] due to endemic infection by Schistosoma. However, this form of the disease has changed in the last 28 years when Schistosoma infection started to decrease by the effective treatment of governmental eradication program of Bilharzia [8]. Bilharzia and Squamous cell carcinoma; both are considered independent poor prognostic factors [19]. Squamous cell carcinoma is a unique histopathological type as it is a rare type with a high percentage (up to 30%) found in few countries where Schistosoma is endemic as Egypt and Sudan [8]. For this reason, studies on this type are limited and treatment also is insufficient. Adjuvant therapy is not usually successful and no novel targeted or immunotherapeutic agents have been identified to provide benefits [3]. Bladder cancer in general, has more incidence in males than females but females have more invasion and worse prognosis than males [11].

This behavior of the disease opens a new way in researches for the possible cause and possible treatment by studying the sex hormones in tumor tissue and association with risk factors and prognosis. Estrogen receptor beta was studied in bladder cancer many years ago with contrary results about its effect on the cancer cell. Some classify it as bad prognostic factor and others as good prognostic factors [17,21]. All previous studies were done for transitional cell carcinoma only. Our aim is to study the Expression of Estrogen receptor beta in SCC and to find the association with the prognostic factors to detect if ER-beta has a role in bladder cancer pathogenesis.

Patients and Methods:

The study included 25 patients with confirmed bladder cancer and was approved by the local ethics committee at SECI with grant number and RIB number: 395. (this was a small sample size due to financial issues as the cost of kites for ER betais expensive exceeding the available range of fund in our institute).

Twenty five formalin-fixed paraffin-embedded tissue blocks were collected from the archived materials of the Pathology Department in the South Egypt Cancer Institute. There were taken either by trans urethral bladder biopsy (TURB) or cystectomy. Clinicopathological parameters such as patient age, sex, histological grade (G), invasion of the bladder (T), lymph node metastasis (LN), lymphovascular invasion (LVI), bilharzial infestation and stage, all were obtained

from the available histopathological reports, and the patient medical record files of SECI.

Immunohistochemistry:

Three µm thick formalin-fixed paraffin-embedded tissue sections were cut and mounted on coated-positive-charged glass slides. Sections were dewaxed in Xylene (for half an hour) and rehydrated through graded alcohols from 100%-70% then washed in Distilled water. Pre-treatment with heat-induced epitope retrieval (HIER) was done using citrate buffer Ph 6 for 20 minutes using microwave then cool for another 20 min. Slides were then washed 2-3 times with phosphate buffer solution (PBS). Blocking of endogenous peroxidase activity was performed using peroxidase blocking reagent (Genemed, Sakura, USA) and incubated 10 minutes at room temperature in a humidity chamber to prevent unnecessary background staining. Polyclonal rabbit anti- Estrogen receptor beta (ER-β) with Catalog no. #YPA1705 primary antibody (Chongqing Biopsies Co., Ltd, China) diluted by 1:100 was applied to the sections and incubated for 1 hour at room temperature. Then the slides were washed 2-3 times using PBS. After washing, immunostaining was performed using a universal staining kit, (Poly HRP/DAB (Ready-To-Use), Genemed, Sakura, USA) following the manufacturer's instructions. The secondary antibody was applied to the slides and incubated for 15 minutes at room temperature, then rinsed and washed with PBS twice, the detection was done by DAB chromogen and substrate for 5 min (Dako, Denmark).Sections were then counterstained using Mayer's hematoxylin (Dako, Denmark) for 7-10 minutes then washed in distilled water, dehydrated in ascending alcohols from 70%-100% then cleared in Xylene and left to dry in air. DPX was applied to each slide and cover was slipped. Sections from the tissue of serous cystadenocarcinoma of the ovary were used as a positive control for evaluation of the ER-β specificity.

Evaluation of ER-beta immunostaining:

All stained tissue was evaluated by a pathologist. The positivity was identified as specific brown nuclear and cytoplasmic staining of tumor cells. Cases< 10% positive cells were considered negative and ≥ 10% were considered positive Figure [1].

Statistical Analysis:

All statistical calculations were done using SPSS (statistical package for the social science; SPSS Inc., Chicago, IL, USA) version 22. Data which are normally distributed were statistically described in terms of mean ± standard deviation (±SD), frequencies (number of cases) and percentages were used for qualitative data. . For comparing categorical data, Chi square ($\chi 2$) test was performed.

Results:

Table 1 shows the clinicopathological characters of the studied cases. 72% of our cases are males. The majority of the tumor (80%) are high grade, while (20%) of all cases, are low grade. 80 % of cases are muscle-invasive. 28% of cases have LN metastasis and the same percentage has LVI. Bilharzial infection were detected in 60% of cases.

Correlation between ER-beta and other studied factors in SCC were explained in Table 2. 36% of our samples are positive for ER-beta. According to the grade; none of the low grade samples shows ER-beta expression when about half of the high grade (45%) are positive for the expression with p-value 0.123. For non-muscle invasive bladder cancer (NMIBC);(60%) of samples are negative and in MIBC; nearly the two-third of samples (65%) are negative with p-value = 1. About LN, we found that in metastatic LN only one sample is positive (14.29%), when in the negative LN 8 samples (44.41%) are positive with p-value 0.355. In bilharzia infection there are 9 samples (60%) are negative and 6(40%) are positive and in bilharzial negative samples there are seven samples (70%) are negative for ER-beta expression and the remaining 3(30%) samples are positive with p-value 0.691.

Table 1: Descriptive analysis of clinicopathological factors in SCC

Variable		Frequency	Percentage
Age (mean ± SD) 40-74		**57.21 ±8.41**	
Sex			
-	Male	18	72%
-	Female	7	28%
Grade			
-	Low grade	5	20%
-	High grade	20	80%
Muscle invasion			
-	Non muscle invasive BC	5	20%
-	Muscle invasive BC	20	80%
LN Metastasis			
-	Positive	7	28%
-	Negative	18	72%
LVI			
-	Positive	7	28%
-	Negative	18	72%
Bilharzial infection			
-	Positive	15	60%
-	Negative	10	40%
Stage			
-	Stage 1	0	0%
-	Stage 2	7	28%
-	Stage 3	16	64%
-	Stage 4	2	8%

SD = standard deviation, BC = bladder cancer, LN = lymph node invasion, LVI = lymphovascular invasion,
* Data are numbers (%) of cases unless otherwise specified.

Table (2): The correlation between ER-beta and characters of SCC tumors

		ER Beta expression for Sq C C cases		p-value
		Negative (n=16) No %	Positive (n=9) No %	
Sex				
-	Male	12 (66.67)	6 (33.33)	0.673
-	Female	4 (57.14)	3 (42.86)	
Grade				
-	Low grade	5 (100.00)	0 (0.00)	0.123
-	High grade	11 (55.00)	9 (45.00)	
T				
-	Non muscle invasive BC	3 (60.00)	2 (40.00)	1.000
-	Muscle invasive BC	13 (65.00)	7 (35.00)	
N				
-	Negative	10 (55.56)	8 (44.44)	0.355
-	Positive	6 (85.71)	1 (14.29)	
LVI				
-	No	10 (55.56)	8 (44.44)	0.355
-	Yes	6 (85.71)	1 (14.29)	
Bilharzial infection				
-	No	7 (70.00)	3 (30.00)	0.691
-	Yes	9 (60.00)	6 (40.00)	
Stage				
-	Stage 1	3 (60.00)	2 (40.00)	
-	Stage 2	1 (25.00)	3 (75.00)	0.326
-	Stage 3	11 (73.33)	4 (26.67)	
-	Stage 4	1 (100.00)	0 (0.00)	

T = tumor invasion, N = lymph node invasion, LVI = lymphovascular invasion, BC =bladder cancer.
* Data are numbers (%) of cases unless otherwise specified.
P-value by Chi-square ($\chi 2$) test.

Discussion:

Bladder cancer is the ninth most common malignancy worldwide [13]. It is a major problem in malignancy due to its high incidence, death rate, and limited lines of treatment. In the last 30 years, before the era of immunotherapy, there was no progress in the treatment of bladder cancer more than platinum-based chemotherapy in which most of our patients are not eligible for, due to impaired kidney function [10]. In the late 1990s/early 2000s, the era of immunotherapy was started and since that time there was marked progress in researches for treatment of many types of cancer including bladder cancer with high burden of cost and side effects. Depending on the observation that bladder cancer has worse prognosis in females despite they have less incidence than males. Our research attempted to study one of the sex hormones in trial to find a gleam of light that can help us to improve treatment and outcome of this disease. We studied ER-beta in bladder cancer to detect if there is an expression and if this expression associated with any risk factors. Almost all of the bladder cancer studies were done for the urothelial carcinoma which is the main histopathological type worldwide. In Egypt, SCC is the second most common type due to Schistosoma infection [7].SCC and bilharzial infection; both considered independent bad prognostic factors [19]. There is insufficient evidence to provide a treatment recommendation for this histopathological type [9]. The main line of treatment for this type is surgery which has better results than Radiotherapy [1].Many studies of bladder cancer focused on the ER-beta expression in transitional cell type (TCC) rather than Squamous cell carcinoma (SCC)which constitute less than 5% in non-endemic countries [6].There were different results about the association with clinicopathological factors, some show ER beta is a bad prognostic factor and suggest treatment by anti-estrogen drugs [12-16].Other results recorded that ER beta is a good prognostic factor [17].To the best of our knowledge, only one study correlates SCC to ER-beta expression inthe bladder cancer [2]. Accordingly, we will compare our results with the corresponding in TCC type.

36% of our cases expressed ER-beta. The percentage of expression varies between studies from 22% -100% as mentioned by Ide et.al. [22] and this variation depends on many technical and pathological factors [23].

In our study, we detected no association between expression of ER-beta in SCC of bladder cancer with any of the clinicopathological factors. This goes on line with some studies and disagrees with others.

There is no association between patients' gender and the expression of estrogen receptors beta .This follows the results of Kaufmann et.al. who studied 88 invasive and 97 non-invasive carcinomas of 101 females and 84 males[16].Accordingly, the difference in disease behavior between males and females can be explained by other genetic and pathological characters of tumors rather than sex hormones.

We also detected no association between the muscle invasion (T) or the grade (G) of the tumor with ER-beta expression and this disagrees with the result of Nam et.al. who concluded that ER-beta expression is associated with increase grade and invasion of the tumor with p-value(<0.001)for tumor invasion and p-value (<0.001) for pathologic grade [20] .This contradiction in results may be attributed to different characters of both studies and small sample sizes of ours. Kontos et.al. concluded different results, as they detected that ER-beta is increasing with decreasing grade of the tumor [17]. Others did not support any of these conclusions as Shen et.al. with p-value =.085 [24] and Tuygun et.al. with p-value 0.441[21].

Regarding the LN metastasis, we supported the results of AlIAA et.al. with p-value = 0.929 [4] that showed no correlation between ER beta and LN metastasis.

We also did not detect any association between ER-beta and LVI (p-value =0.430). This was different from the results of Kauffman et.al. with p-value =0.008[15] and ALIAA et al. with P-value=0.041 [4] as both studies detected that ER-beta expression associated

with LVI positivity. This could be explained by the difference in the histopathological types between their study and ours, as they studied TCC only while we studied SCC type.

The same for bilharzial infection. We detected no difference between bilharzial and non-bilharzial associated bladder cancer in ER-beta expression with p-value=0.691 which supports the results of Aboushousha et.al.[2].

Regarding stage at presentation, there is no correlation with ER-beta in SCC with p-value=0.32, and this agrees with the results of Kashiwagi et al. Who studied ER beta inupper urinary tract tumor with p-value =0.831[14].These different results may be attributed to the small size of our sample, different histopathological types, and also bilharzial infection that was detected in our cases.

Accordingly, another larger multicenter study is recommended to evaluate ER-beta in SCC and if it really has no clinical impact even with this percentage of expression.

Conclusion:

ER-beta is expressed in 36% of SCC of bladder cancer. There is no statistically significant association with any clinical or pathological characters of the cases and this is contrary to transitional cell carcinoma (TCC) type that detects ER-beta as a bad prognostic factor in most of the studies. Another study with large numbers of patients is recommended to confirm these results.

List of abbreviations

ER beta	= Estrogen receptor beta
SCC	= Squamous cell carcinoma
TCC	= Transitional cell carcinoma
SECI	= South Egypt Cancer Institute
(TURB)	= Trans urethral resection bladder
G	= Grade
T	= Muscle invasion
MIBC	= Muscle invasive bladder cancer
NMIBC	= Non muscle invasive bladder cancer
LN	= Lymph node metastasis
LVI	= Lymphovascular invasion

References:

1. Abdel-Rahman O. **Squamous Cell Carcinoma of the Bladder: A SEER Database Analysis**. Clin Genitourin Cancer. 2017 Jun;**15(**3):e463-e468.
2. Aboushousha T, Hammam O, Abdelnasser A, et al. **Possible role of RAGE and ER expression in therapy of bladder cancer. Possible role of RAGE and ER expression in therapy of bladder cancer**. Cancer Rep Rev 2018, **2**. DOI: 10.15761/CRR.1000173.
3. Alanee S, Alvarado-Cabrero I, Murugan P, et al. **Update of the International Consultation on Urological Diseases on bladder cancer 2018: non-urothelial cancers of the urinary bladder.** World J Urol . 2019 Jan;**37**(1):107-114.
4. El-Nady M, Atef A. El-Shenawy H, et al. **Immunohistochemical Expression of Androgen and Estrogen Receptors and their Prognostic Significance in Urothelial Carcinoma of the Urinary Bladder**. Med. J. Cairo Univ. 2018, **86**(1):305-10.
5. Antoni S, Ferlay J, Soerjomataram I, et al. **Bladder Cancer Incidence and Mortality: A Global Overview and Recent Trends**. Eur Urol. 2017 Jan;**71**(1):96-108.
6. Dahm P, Gschwend JE. **Malignant non-urothelial neoplasms of the urinary bladder: a review**. Eur Urol. 2003 Dec;**44**(6):672-81.
7. Fedewa SA 1, Soliman AS, Ismail K, et al. **Incidence analyses of bladder cancer in the Nile delta region of Egypt.** Cancer Epidemiol. 2009 Oct;**33**(3-4):176-81.
8. Felix AS, Soliman AS, Khaled H, et al. **The changing patterns of bladder cancer in Egypt over the past 26 years**. Cancer Causes Control. 2008 May;**19**(4):421-9.
9. Ferlay J, Soerjomataram I, Ervik M, et al. France: International Agency for Research on Cancer; 2013. **Cancer Incidence and Mortality Worldwide**: IARC CancerBase 2013, **10**(3), 17-21.
10. Grayson M. **Bladder cancer**. Nature. 2017 Nov 8;**551**(7679):S33.
11. Horstmann M, Witthuhn R, Falk M, et al. **Gender-specific differences in bladder cancer: a retrospective analysis**. Gend Med. 2008 Dec;**5**(4):385-94.
12. Hsu I, Vitkus S, Da J, et al. **Role of oestrogen receptors in bladder cancer development** Nat Rev Urol. 2013 Jun;**10**(6):317-26.
13. Jemal A, Bray F, Center MM, et al. **Global cancer statistics**. CA Cancer J Clin. Mar-Apr 2011;**61**(2):69-90.
14. Kashiwagi E, Fujita K, Yamaguchi S, et al. **Expression of steroid hormone receptors and its prognostic significance in urothelial carcinoma of the upper urinary tract**. Cancer Biol Ther. 2016 Nov;**17**(11):1188-1196.
15. Kauffman EC, Robinson BD, Downes M, et al. **Estrogen receptor-β expression and pharmacological targeting in bladder cancer**. Oncol Rep. 2013 Jul;**3**0(1):131-8.
16. Kaufmann O, Baume H, Dietel M. **Detection of oestrogen receptors in non-invasive and invasive transitional cell carcinomas of the urinary bladder using both conventional immunohistochemistry and the tyramide staining amplification (TSA) technique**. J Pathol. 1998 Oct;**18**6(2):165-8.
17. Kontos S, Kominea A, Melachrinou M, et al. **Inverse expression of estrogen receptor-beta and nuclear factor-kappaB in urinary bladder carcinogenesis**. Int J Urol. 2010 Sep;**17**(9):801-9.
18. Kyritsi F, Loffredo CA, Zheng YL, et al. **Urinary Bladder Cancer in Egypt: Are There Gender Differences in Its Histopathological Presentation**? Adv Urol. 2018 Mar 13;**2018**:3453808.

19. Nagy A, Darweish H, Hamdey H, et al. **Factors Affecting Survival in Egyptian Patients Suffering from Urinary Bladder Cancer: A Multicenter Retrospective Study**. J Cancer Sci Ther 2018. **10**(7), 031-035.
20. Nam JK, Sung Park W, Lee SD, et al. **Prognostic value of sex-hormone receptor expression in non-muscle-invasive bladder cancer**. Yonsei Med J. 2014 Sep;**55**(5):1214-21.
21. Tuygun C, Kankaya D, Imamoglu A, et al. **Sex-specific hormone receptors in urothelial carcinomas of the human urinary bladder: a comparative analysis of clinicopathological features and survival outcomes according to receptor expression**. Urol Oncol. Jan-Feb 2011;**29**(1):43-51.
22. Ide H, Inoue S, Miyamoto H. **Histopathological and prognostic significance of the expression of sex hormone receptors in bladder cancer: A meta-analysis of immunohistochemical studies**. PLoS One. 2017 Mar 31;**12**(3):e0174746.
23. Godoy G, Gakis G, Smith CL, et al. **Effects of Androgen and Estrogen Receptor Signaling Pathways on Bladder Cancer Initiation and Progression**. Bladder Cancer. . 2016 Apr 27;**2**(2):127-137.
24. Shen SS, Smith CL, Hsieh JT, et al. **Expression of estrogen receptors-alpha and -beta in bladder cancer cell lines and human bladder tumor tissue**. Cancer 2006 Jun 15;**106**(12):2610-6.