Hormonal receptors expression in urinary bladder cancer

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Abstract:

Background: Urinary bladder cancer (UBC) is one of the top ten cancer types worldwide, with an annual incidence of 550,000 new cases. The incidence varies according to geographical regions, the highest rates were observed in Europe, North America, and Egyptian Syrian, and Turkish men. The lowest incidence was in Mexico, some Middle Eastern, Central Asian countries, and Sub-Saharan Africa. Estrogen receptor (ER) and progesterone receptor (PR) play an important role during tumorigenesis and the progression of several malignancies.

Objective: There is a need to evaluate hormonal receptors in UBC as this may help to play a role in hormonal treatment in UBC.

Methods: We investigated the expression of ER and PR in 168 UBC specimens, and its relation with clinicopathological features and survival data.

Results: We found that 33.3% of the included patients were hormone positive, (ER 23.2% and PR 20.8%). The ER status differed significantly according to the pathology, grade, and T stage. The highest prevalence of positive ER was observed in TCC pathology, grade I tumors, and T 1 or 2 stage tumors. Only the T stage had a significant relationship with PR status and was higher in T 1 or 2 tumors. The hormonal status differed significantly according to the pathology and T stage. We did not find a significant effect of ER, PR, or total HR status on OS.

Conclusions: There is a significant relation between ER status and pathology type, differentiation grade, and tumor stage. Further studies are needed to evaluate the role of hormonal treatment in the management of UBC.

Keywords: Bladder cancer, ER, PR, Hormonal Receptors

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Introduction:

Bladder cancer is one of the top ten cancer types in the world, with annually 550,000 new cases approximately. [1] 3.0% of all new cancer diagnoses and 2.1% of all cancer deaths are due to urinary bladder cancer (UBC). [2] In 2018 200,000 patients died with UBC Worldwide 2018. [3]

UBC incidence varies significantly according to geographical regions, with highest rates in Europe and North America, also in Egyptian Syrian and Turkish males. Lower rates are seen in South-East Asia (except for Japan) in Latin America and Northern Africa. The lowest incidence countries are Mexico, some Middle Eastern, Central Asian countries and Sub-Saharan Africa. [4]

Transitional cell carcinoma (TCC) constitutes about 90% of bladder cancers in industrialized countries. In developing countries (particularly Middle East and Africa), the majority of UBC cases are squamous cell carcinoma (SCC). The highest incidence of SCC was found schistosomal-endemic areas, like Sudan and Egypt as it ranges between two-thirds to three-quarters of UBC cases. Some studies from Egypt have shown a reversal of this ratio due to better schistosomiasis control; also increased smoking incidence may have contributed to the shift in Egypt. While in other African countries the association remains unchanged. [5,6,7]

Systemic chemotherapy is the standard approach as an initial treatment for locally advanced inoperable or metastatic urothelial malignancies. Even with high

initial response rates, the median survival with multi-agent chemotherapy remains around 15 months [8-9]. Second-line chemotherapy played a limited role to improve these results. The use of immune checkpoint inhibitors offers an additional option for patients progressing after their initial systemic therapy and those who are not candidate for chemotherapy. [10]

The expression of estrogen receptor (ER) and progesterone receptor (PR) plays an important role during tumorigenesis and progression of several malignancies, offering a rationale for hormonal treatment as a targeted therapy. [11] In breast cancer, ER and PR status are established prognostic factors in the identification of patients who may benefit from hormonal treatment. UBC cell lines express ER, and in vitro studies have shown that anti-estrogens can inhibit UBC growth. [12]

There is increasing evidence indicating the roles of sex hormone receptors in the development and progression of UBC. These included both the stimulatory and inhibitory effects of estrogens and selective ER modulators on urothelial carcinoma cell growth via the ERα and/or ERβ. [13] Previous attempts for assessment of AR (androgen receptors), ERα and/or ERβ expression in UBC tissue specimens leads to conflicting outcomes, including receptor expression status and its relation to tumors histopathological characteristics. Earlier studies showed the presence of ER in 12 – 33% of UBC tissues. ER expression rates were especially higher in high-grade and/or invasive tumors. Furthermore, little is known regarding the prognostic significance of receptor expression in UBC patients. [14] The potentially toxic chemotherapy regimens and expensive immunotherapy regimens, especially in developing countries for the treatment of advanced urinary bladder cancer push us to think in assessment of estrogen receptors and Progesterone receptors, which may allow to use a relatively safe and cheap treatment (Hormonal treatment) for patients with hormonal receptors positive urinary bladder cancer.

Aim of the work:

The primary end point: To assess the expression of estrogen and progesterone receptors in urinary bladder cancer specimens. The secondary end points: to study the correlation of the ER and PR status with tumor, patients' characteristics and survival data.

Methods:

This is a retrospective study for a group of cancer bladder patients presented in Sohag Cancer Center or Sohag University Hospital during 2/2012 to 2/2016. We investigated the expression of estrogen (ER) and progesterone receptors (PR) for 168 urinary bladder cancer specimens by immunohistochemistry.

We retrospectively extract the clinic-pathological and survival data from the available medical files for this group of patients. We used the American Joint Committee on Cancer (AJCC) TNM staging system for Urinary Bladder cancer (2017). We evaluated the relationships between the expression of these receptors and the clinicopathological features and survival data for this group of patients. Disease-free survival (DFS): calculated from the date of complete response (CR) to a radical radiotherapy course, radical radiotherapy concurrent with chemotherapy, neoadjuvant chemotherapy, or intravesical instillation of BCG or after radical cystectomy (only for patients who achieved CR or underwent radical cystectomy) to the date of recurrence / death whichever comes first. Progression-free survival (PFS) calculated as the length of time from urinary bladder cancer diagnosis, during and after the treatment of it that a patient lived without disease progression to the date of progression or death whichever comes first. Overall Survival (OS): calculated from the time of diagnosis of UB cancer up to the time of death. Patients alive or lost to follow-up were censored.

Formalin-fixed paraffin-embedded tissue blocks of 168 tumors obtained from either transurethral resection (n=120) or cystectomy (n=48) were retrieved for the study. Clinical data, postoperative therapeutic regimens and follow-up data were obtained from the patients` clinical files and histological sections of the tumors were reevaluated for different histopathological parameters and pathological staging.

Immunohistochemistry:

Tissue sections of four micrometer-thickness were de-deparaffinized and rehydrated before washing in running water. Endogenous peroxidase was blocked by incubation for 10 minutes in a dual endogenous enzyme-blocking solution (Dako Code K4065) followed by washing thoroughly in running water. Antigen epitopes were unmasked by boiling tissue sections in 10 mM citrate buffer, pH 6.0 in a microwave at high power. After cooling to room temperature; the sections were washed twice in phosphate-buffered saline (PBS) pH 7.6. Tissue sections were incubated with either mouse monoclonal anti-human ER-α (Dako, clone 1D5, code M7047), or mouse monoclonal anti-human PR (Dako, clone 636, code M3569) for 60 minutes at room temperature; followed by proper washing using PBS. Tissue sections were incubated for 30 minutes at room temperature with polymer-conjugated goat anti-mouse secondary antibody labeled with peroxidase enzyme (Dako, code K4065). The sections were washed properly using PBS and incubated with 3, 3′-diaminobenzidine tetrahydrochloride (DAB) solution (Dako, code K4065) for 5-10 minutes till the development of brown deposit. The sections were counterstained with hematoxylin, dehydrated and mounted as usual. Sections of hormone-positive breast cancer served as positive control and replacement of primary antibodies with BPS served as a negative control for immunohistochemistry steps. The expression of ER and PR was evaluated based on the percentage of positive cells. Both nuclear and cytoplasmic staining were considered during evaluation and final score ranged between 0 and 100%. Cells in four high-power fields were counted and the percentage of positive cells was calculated accordingly. The intensity of the immunoreaction for positive cases was ranked as weak,

moderate and strong expression. We used the Allred scoring system for estrogen receptor and progesterone receptor. Which combine the proportion score (the percentage of stained cells) with the intensity score (The intensity of the staining). Scores of 0 and 2 are considered negative. Scores of 3-8 are considered positive.

Statistical analysis:

Categorical data were described as number and percentage and continuous variables as mean and standard deviation. The significance of differences in continuous variable between two groups was tested using T-test. Chi-squared test was used to test the significance of differences in the distribution of categorical variables between groups. The Kaplan-Meier method was used to estimate progression-free survival (PFS) and disease-free survival (DFS) and the Log rank test was used to compare survival curves. Statistical analysis was performed using MedCalc® Statistical Software version 20.014 (MedCalc Software Ltd, Ostend, Belgium).

Ethical approval:

Ethical approval for conduction of this research was obtained from the Research Ethical Committee, Faculty of Medicine, Sohag University (approval number: Soh-Med-21-04-40). All procedures followed were in accordance with the ethical standards of the responsible committee of the faculty of medicine, Sohag University (Soh.Med-20.12.16), and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was not required in this retrospective study and data obtained from archived tissue and patient medical record.

Results:

We investigated the expression of estrogen and progesterone receptors in the pathology specimens of 168 urinary bladder cancer, and evaluated the relationships between their expression and the available clinicopathological features and the survival data of this group of patients. Medical files of 43 patients from the included group were not available, so no survival data about those patients, Participants' ages ranged between 35 and 90 years (Mean = 59 and Standard Deviation = 10.71). One hundred and thirty-three patients were males and 35 were females. 27 patients from the study group received neoadjuvant chemotherapy. No one received neoadjuvant radiotherapy.31 patients received postoperative (adjuvant radiotherapy), and 26 patients received adjuvant chemotherapy (7 patients received MVAC regimen for 4 cycles, 9 patients received Gemcitabine / Cisplatin for 4 cycles and 10 patients received Gemcitabine / Carboplatin for 4 cycles)

Regarding tumor characteristics (table 1). We found that 33.3% of the included patients were hormone positive (ER and or PR positive), ER was positive in 23.2% and PR was positive in 20.8% of the included patients. ER and PR positivity intensities are shown in tables (2) and (3).

Table 1: Tumor characteristics

Variable	Number of patients	Percentage
Pathology		
TCC	122	72.6%
SqCC	32	19%
Undiff	10	6%
Adenocarcinoma	3	1.8%
Spindle cell tumor	1	0.6%
Grade		
I	14	8.3%
II	37	22.1%
III	117	69.6%
T		
1	12	9.5%
2	25	19.8%
3	77	61.1%
4	12	9.5%
N		
0	79	62.7%
1	32	25.4%
2	15	11.9%
M		
0	113	89.7%
1	13	10.3%

TCC (Transitional Cell Carcinoma),
SqCC (Squamous Cell Carcinoma),
Undiff (Undifferentiated Carcinoma).

Table 2: ER intensity

ER intensity	Number of patients	Percentage
moderate	9	5.4%
negative	129	76.8%
strong	2	1.2%
weak	28	16.7%
Total	168	100.0%

ER (Estrogen Receptor)

Table 3: PR intensity

PR intensity	Number of patients	Percentage
moderate	11	6.5%
negative	133	79.2%
strong	3	1.8%
weak	21	12.5%
Total	168	100.0%

PR (Progesterone Receptor)

The relationship between the ER, PR and HR statuses and the studied variables is illustrated in table (4). The ER status differed significantly according to the pathology, grade and T stage. The highest prevalence of positive ER was observed in TCC pathology (P value 0.02), grade I tumors (P value 0.041) and T 1 or 2 tumors (P value 0.039). There was no significant relationship between the ER status and the other variables. Only T stage had a significant relationship with PR status and was higher in T 1 or 2 tumors (P value 0.016). The hormonal (ER/PR) status differed significantly according to the pathology and T stage.

Table (4): The relationship between ER, PR and ER/PR expression and the studied variables

Variable	**ER**			**PR**			**ER/PR**		
	-ve	**+ve**	***P***	**-ve**	**+ve**	***P***	**-ve**	**+ve**	***P***
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
Age	60 (11.1)	58 (9.1)	0.303	59 (11.1)	61.5 (9.2)	0.219	59.3 (11.2)	59.9 (9.9)	
	n (%)	*n* (%)		*n* (%)	*n* (%)		*n* (%)	*n* (%)	
Sex									
Female	24 (68.6)	11 (31.4)	0.197	28 (80)	7 (20)	0.892	21 (60)	14 (40)	0.349
Male	105 (78.9)	28 (21.1)		105 (78.9)	28 (21.1)		91 (68.4)	42 (31.6)	
Pathology									
TCC	87 (71.3)	35 (28.7)	**0.02**	92 (75.4)	30 (24.6)	0.138	74 (60.7)	48 (39.3)	**0.015**
SqCC	30 (93.7)	2 (6.2)		29 (90.6)	3 (9.4)		28 (87.5)	4 (12.5)	
Other	12 (85.7)	2 (14.3)		12 (85.7)	2 (14.3)		10 (71.4)	4 (28.6)	
Grade									
I	7 (50)	7 (50)	**0.041**	11 (78.6)	3 (21.4)	0.318	7 (50)	7 (50)	0.273
II	28 (75.7)	9 (24.3)		26 (70.3)	11 (29.7)		23 (62.2)	14 (37.8)	
III	93 (80.2)	23 (19.8)		95 (81.9)	21 (18.1)		81 (69.8)	35 (30.2)	
T									
1 or 2	23 (62.2)	14 (37.8)	**0.039**	24 (64.9)	13 (35.1)	**0.016**	16 (43.2)	21 (56.8)	**0.002**
3 or 4	71 (79.8)	18 (20.2)		75 (84.3)	14 (15.7)		64 (71.9)	25 (28.1)	
N									
0	57 (72.2)	22 (27.8)	0.604	62 (78.5)	17 (21.5)	0.827	46 (58.2)	33 (41.8)	0.281
1	26 (81.2)	6 (18.8)		26 (81.2)	6 (18.8)		23 (71.9)	9 (28.1)	
2	11 (73.3)	4 (26.7)		11 (73.3)	4 (26.7)		11 (73.3)	4 (26.7)	
M									
0	84 (74.3)	29 (25.7)	0.84	90 (79.6)	23 (20.4)	0.388	71 (62.8)	42 (37.2)	0.651
1	10 (76.9)	3 (23.1)		9 (69.2)	4 (30.8)		9 (69.2)	4 (30.8)	
Response									
CR	36 (67.9)	17 (32.1)	0.234	40 (75.5)	13 (24.5)	0.888	28 (52.8)	25 (47.2)	0.087
PR	21 (80.8)	5 (19.2)		20 (76.9)	6 (23.1)		19 (73.1)	7 (26.9)	

ER (Estrogen Receptor), PR (Progesterone Receptor), CR (Complete Response), PR (Partial Response), T (Primary Tumor Stage), N (regional Nodal status), M (Presence or no Metastasis), TCC (Transitional Cell Carcinoma) , SqCC (Squamous Cell Carcinoma)

Survival statistics:

DFS was calculated for 89 patients of the whole study population (who achieved CR or after radical surgery) the 3-years DFS and 5-years DFS were 82% and 50% respectively (figure 1), this means 73 patients (from the total 89 patients) disease-free survived for 3 years and 44 patients (from the total 89 patients) disease-free survived for 5 years. There was no significant effect of the ER PR status or total HR status on DFS (figure 2,3, and 4). PFS was calculated for 125 patients of the whole study population 3 years and 5 years PFS were 80% (N = 100 from 125 patients) and 57% (N = 71 from 125 patients) (figure 5). There was no significant relation between ER, PR or total HR and the PFS figures (6,7, and 8). The 3 years and 5-years OS were 83% and 58% respectively (figure 9). The median OS was 63 months. We did not find a significant effect of ER, PR or total HR status on OS (figure 10).

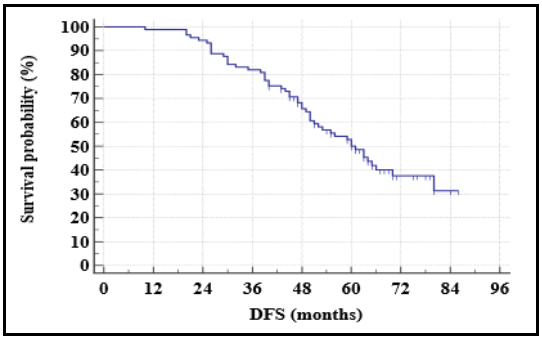

Figure (1): Disease Free Survival (DFS), Patients (N = 89), UB cancer, Mean 60.119 (95% CI 55.180 to 65.058)

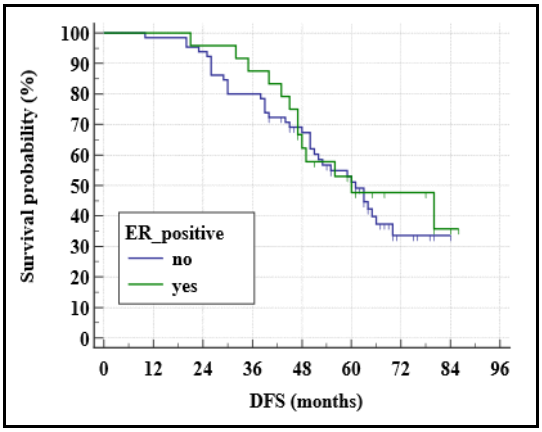

Figure (2): Effect of the ER status on DFS, UB cancer, Patients (n = 65 negative ER [no] &24 positive ER [yes]), P = 0.6058, [mean of negative ER 58.502 - 95% CI52.812 to 64.192], [mean of positive ER 63.380- 95% CI 54.541 to 72.220]. ER (Estrogen Receptors), DFS (Disease Free Survival).

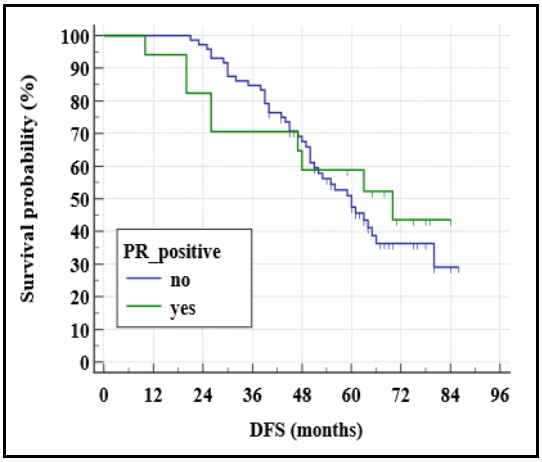

Figure (3): Effect of PR status on DFS, UB cancer, Patients (n = 72 negative PR [no] &17 positive PR [yes]), P = 0.6714, [mean of negative PR 60.287- 95% CI 55.081 to 65.493], [mean of positive PR 58.407- 95% CI 45.332 to 71.483]. PR(Progesterone Receptors) , DFS (Disease Free Survival).

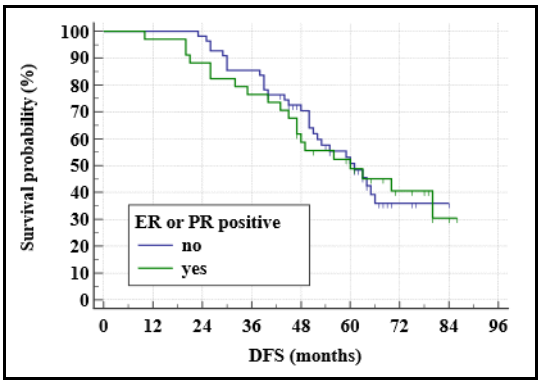

Figure (4): Effect of HR status on DFS, UB cancer, Patients (n = 55 negative HR [no] & 34 positive HR [yes]), P = 0.8988, [mean of negative HR 60.429- 95% CI 54.657 to 66.200], [mean of positive HR 58.838- 95% CI 50.337 to 67.338]. HR (Hormonal Receptors = ER and or PR), DFS (Disease Free Survival).

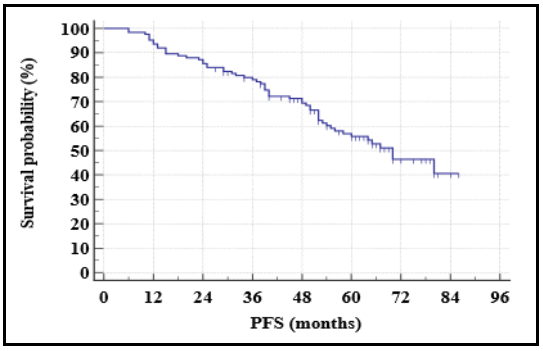

Figure (5): PFS (Progression Free Survival), Patients (N = 125), UB cancer, Mean 61.218 (95% CI 56.284 to 66.153)

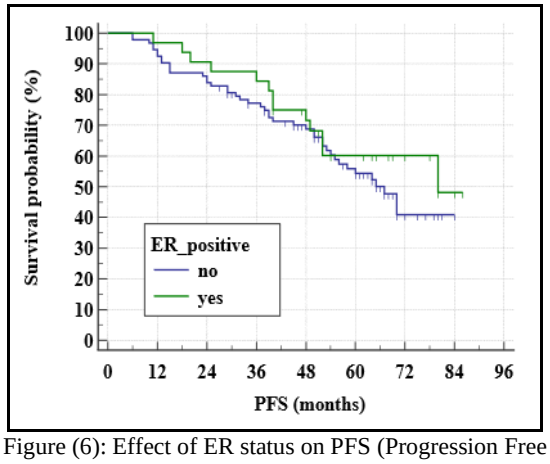

Figure (6): Effect of ER status on PFS (Progression Free Survival), UB cancer, Patients (n = 93 negative ER [no] &32 positive ER [yes]), P = 0.3611, [mean of negative ER 58.988- 95% CI 53.351 to 64.624], [mean of positive ER 65.650- 95% CI 56.693 to 74.608]. ER (Estrogen Receptors).

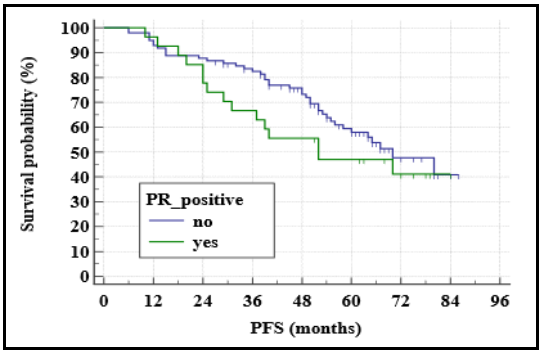

Figure (7): Effect of PR on PFS (Progression Free Survival), UB cancer,
Patients (n = 98 negative PR [no] &27 positive PR [yes]), P = 0.2897, [mean of negative PR 62.791- 95% CI 57.305 to 68.277], [mean of positive PR 54.590- 95% CI 43.969 to 65.212]. PR (Progesterone Receptors).

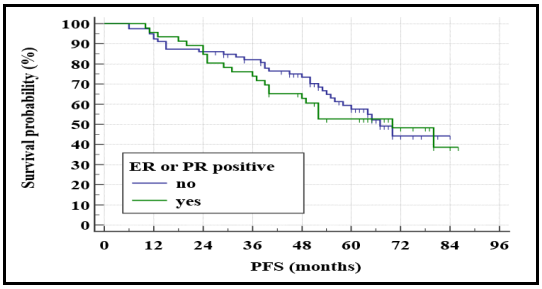

Figure (8): Effect of total HR on the PFS (Progression Free Survival), UB cancer,
Patients (n = 79 negative HR [no] & 46 positive HR [yes]), P = 0.6441, [mean of negative HR 61.353- 95% CI 55.344 to 67.361], [mean of positive HR 59.547- 95% CI 51.484 to 67.610]. HR ((Hormonal Receptors = ER and or PR).

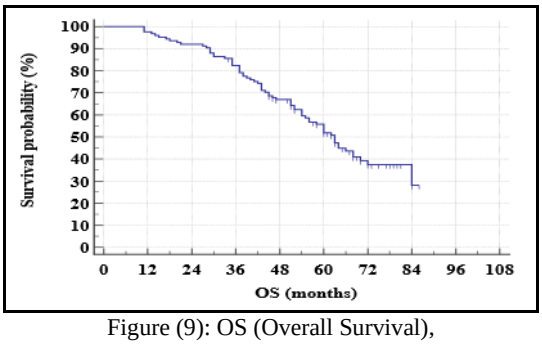

Figure (9): OS (Overall Survival),
Patients (N = 125), UB cancer, Mean 60.515 (95% CI 56.232 to 64.799)

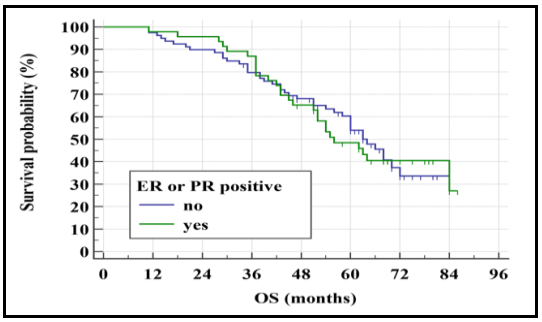

Figure (10): Effect of total HR on the OS (Overall Survival), UB cancer,
Patients (n = 79 negative HR [no] & 46 positive HR [yes]), P = 0.9186, [mean of negative HR 59.611- 95% CI 54.264 to 64.958], [mean of positive HR 60.660- 95% CI 53.956 to 67.363]. HR ((Hormonal Receptors = ER and or PR).

Discussion:

Several studies, either in vitro or in vivo, have shown the functions of ER signals in UBC. This includes the stimulatory and inhibitory effects of estrogens and selective ER modulators on urothelial carcinoma cell growth [15,16,17]. Some studies documented ER positivity in 12 – 33% of UBC cases tissues. The rates were higher in high-grade and/or invasive tumors. [18,19,20]. In other studies, ERα signals have been detected (via IHC) in tissue specimens only in a small UBC patient subset (e.g. 1–5%) [21, 22, 23]. In Miyamoto H et al, ERα was positive in 27% of bladder tumors when examined by IHC [24]. This is matched with our study where we found 23.2% of the tumor specimens positive for ER. And in the previous study A significantly higher expression of ER-alpha in lymph node metastases compared to primary tumors was also noted. In our study, we didn't perform expression of hormonal receptors in primary tumors versus lymph nodes separately. Also in the previous study ER-alpha levels were significantly reduced in high-grade and metastatic tumors compared to low-grade and non-metastatic tumors. This is matched with our study results.

In a study conducted by Eiji Kashiwagi, et al, the expression of ERα in urothelial tumor tissues was positive in 18% of the examined cases [25]. In vitro and animal studies have suggested the benefit of tamoxifen (as an anti-estrogen) in reducing UBC incidence following carcinogen exposure. [26]

The role of progesterone receptor (PR) A in UBC, which was expressed in the squamous epithelium of the urethra, has not been clearly understood. [27] In Eiji Kashiwagi, et al, found that the expression of PR in urothelial tumor tissues was positive in 16% (16 patients), whereas in our study, the expression of PR was 20.8% (35 patients).

We found in our study that ER status differed significantly according to the pathology, grade and T stage, with the highest prevalence of positive ER observed in TCC pathology, grade I tumors and T 1 or 2 tumors. There was no significant relationship between the ER status and the other variables. Only T stage had

a significant relationship with PR status and was higher in T 1 or 2 tumors. While in that study of Eiji Kashiwagi, et al, they failed to show significant differences in the expression of any of the steroid hormone receptors between low-grade/superficial vs. high-grade/muscle-invasive tumors. [28]

As we found in our study, some previous studies have shown a non-significant prognostic effect of ERα expression in patients with urothelial tumors. [28–30]. A study conducted by Kashiwagi E et al demonstrated that patients with pT3-4 upper urinary tract urothelial carcinoma negative for both ERα and progesterone receptor (PR) had a significantly lower risk of cancer-specific mortality, compared with those showing ERα and/or PR positivity. [31]

Conclusion:

Expression of ER and PR in urinary bladder cancer specimens was 23.2% and 20.8% respectively. There was a significant relation between ER status and both pathology and differentiation grade. No statistically significant relation between ER and PR with the patient's characteristics nor the survival parameters. Further studies are needed to evaluate the role of hormonal treatment in management of urinary bladder cancer.

Competing interests:

All authors declare no conflict of interest

Authors' contributions:

All authors contributed to the work as follow:

- First: author: Research idea, data collection data analysis and manuscript writing
- Second author: pathology lab work, pathology data collection.
- Third author: patient diagnostic workup, data review.
- Fourth author: patient diagnostic workup, data review
- Fifth author: data analysis and manuscript writing, review and manuscript submission.

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