Significance of HER2 expression in urinary bladder cancer: single institutional data

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Abstract:

Background: Targeted and immunotherapy got approval to treat locally advanced and/or metastatic urinary bladder (UB) cancer in specific situations. Human epidermal growth factor receptor 2 (HER2) expression is certified in UB cancer but with controversial results regarding the impact of targeted therapies on survival. This study explored expression of HER2 protein in UB cancer patients in our locality and its correlation with clinical outcomes.

Methods: One hundred-thirty two samples of UB cancer collected and tested for HER2 receptor expression using immunohistochemistry (IHC) analysis. Together with patients medical records from 2012 through 2016 used analyze its significance with tumor characteristics and investigate its effect on DFS, PFS and OS. This work was conducted at Sohag University Hospital and Sohag Cancer Center. Data was analyzed using STATA intercooled version 16 for clinic-pathological associations.

Results: HER2 expression was positive in 65% of samples; only pathological subtype had a significant association with HER expression intensity (p= 0.001). No significant association found between HER2 expression and other variables as regards age, tumor size, nodal spread or stage. As regards OS; HER2 positive expression associated with significant longer cumulative OS (p=0.006) and in multivariate Cox-regression analysis; it was the only factor associated with statistically significant longer OS (p=0.04; HR=0.43). No significant association found in the cumulative or Cox analysis for DFS and PFS (p= 0.79 and p= 0.08 respectively).

Conclusion: in this work; HER2 expression was higher in bladder cancer cases especially in TCC. Also, patients with HER2 positive protein expression had a significant longer OS with probability and regression positive effect. No significant effect on DFS or PFS in the probability or Cox analysis of HER2 expression. However, these findings mandate larger prospective studies with anti-HER2 agents included in the management of these patients.

Key words: HER2 expression, Bladder Cancer

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Introduction:

Bladder cancer is the third most common cancer in Egypt and the second among males as estimated by Global Cancer Observatory (GLOBOCAN) in December 2020, with an incidence of 10,655 new cases [1, 2] predominantly in males with 4:1 male to female ratio [3,4]. Also, it is the third lethal cancer with 6170 deaths (~7%) of 89,042 total deaths for all cancers [1].

Invasive and/or metastatic bladder cancer accounts for nearly one third of newly diagnosed cases. [5] Chemotherapy is mostly the systemic treatment of choice for this category. However, it is not a curative and other therapies like the targeted therapy or immunotherapy needed. [6,7]

HER2 overexpression has been identified in bladder cancer [8] with variable expression rates ranged between 31 and 65.5% of samples [9, 10 https://www.spandidos-publications.com/10.3892 /mco.2018.1786 - b75-mco-0-0-1786, 11, 12]. A previous study identified that the 5-year disease-free survival rate decreased from 48.5% in HER2-negative patients to 9.7% in those who were HER2-positive [9]. Skagias et al [13], also identified that HER2 expression was correlated with decreased disease-specific survival (P=0.002) and overall survival rates (P=0.025).

Different molecular testing recommended to be performed as early as diagnosis of advanced bladder cancer has been established, this needed to facilitate

treatment decision- making and to prevent delays in administering later lines of therapy. Yet, among malignancies with certified HER2 overexpression, bladder cancer has limited Anti-HER2 options because of controversies regarding the impact of targeted therapies on patient's survival. [14]

Egypt is a developing country, [15] and cost-effective treatments are highly favored by the national healthcare provider, so, expensive therapies like targeted therapy or immunotherapy can be an obstacle in treating bladder cancer. However, September 2018 was a new hope as the government announced the initiative of President Abdel Fatah el-Sisi "100 Million Healthy Lives," to eradicate Hepatitis C which expanded to include cancer patients care with targeted treatment like Anti-HER2 trustuzumab in breast cancer cases [16], so, targeting HER2 pathway in bladder cancer may be effective in the near future.

This study conducted to test HER2 expression level among newly diagnosed bladder cancer patients in our locality and correlation with different pathological and clinical characteristics was analyzed as it may be possible targetable mechanism for UB cancer treatment in the near future. To our knowledge; it is the first study to investigate this matter in Upper Egypt.

Patients and Methods:

Patient`s and tissue samples:

Tumor samples of bladder cancer patients treated at Sohag University Hospital and Sohag Cancer Institute were included in this study after obtaining ethical approval from institutional Research Ethical Committee (approval number: Soh-Med 21-4-40). Samples were obtained by either transurethral resection (TUR) or cystectomy in eligible patients. Clinical and follow up data obtained from the patients` medical records and histological sections of the tumors were evaluated.

Immunohistochemistry

Formalin fixed paraffin embedded blocks of tumor tissue were sectioned at 4µm on coated pre-labeled slides. The sections were de-deparaffinized, rehydrated and washed in tap water. To block tissue peroxidase; the sections were incubated in dual enzyme blocking solution (Dako Code K4065) for 10 min; then washed in running tap water. For epitopes retrieval; the tissue sections were boiled in citrate buffer (10mM, pH 6.0) using a microwave at high power. After cooling to room temperature; tissue sections were washed in phosphate-buffered saline (PBS) pH 7.6; then incubated with primary rabbit poly-clonal anti-human c-erbB-2 protein (Dako, code A0485) antibody for overnight at 4°C temperature. (Dako, clone 1D5, code M7047). After proper washing with PBS; the tissue sections were incubated for 30 min at room temperature with EnVision® + Dual Link System-HRP; a polymer conjugated goat anti-rabbit and anti-mouse secondary antibody (Dako, code K4065). The sections were washed with PBS and incubated with DAB solution for 5-10 min. The sections were counterstained with hematoxylin, dehydrated and mounted. Sections of HER2 positive breast cancer were used as positive control and replacement of the primary antibodies with BPS served as negative control for the procedure. Staining was scored according to the same standard criteria used in breast cancer.

Immunohistochemistry scoring and statistical analysis

HER2 expression was evaluated as percentage of positive cells among tissue sections and final score ranged between 0 to 100%. Cells in four high power fields (x40) of tumor tissue were evaluated and the final score was the mean percentage among all fields. Both membranous and cytoplasmic staining was considered during histological evaluation of HER2 expression. The intensity of immunoreaction for positive cases was ranked as weak, moderate and strong expression. For statistical purpose; negative and weak staining considered as one group while moderate and strong staining considered the other comparative group.

Consent: written informed.

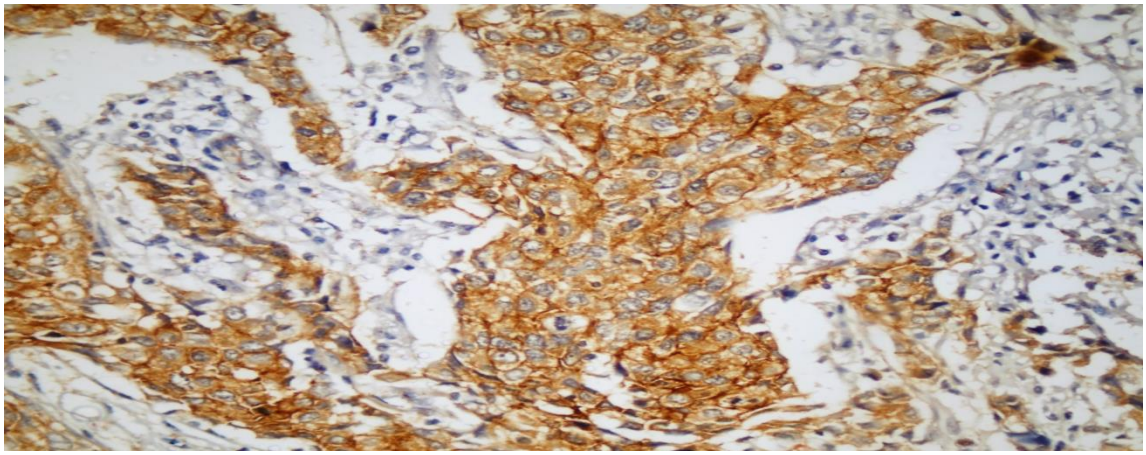
Statistical analysis

Data was analyzed using STATA intercooled version 16. Quantitative data was represented as mean and standard deviation or median and range. Data was analyzed using independent student T test for normally distributed data. Data was analyzed using Mann-Whitney if data is not normally distributed. Qualitative data was presented as number and percentage and compared using Chi square test or fisher exact test. The log-rank test was performed to evaluate significant differences between survival curves of different variables. Hazard ratios (HR) with 95% confidence intervals (CI). Graphs were produced by STATA program. P value was considered significant if it was less than 0.05.

Results:

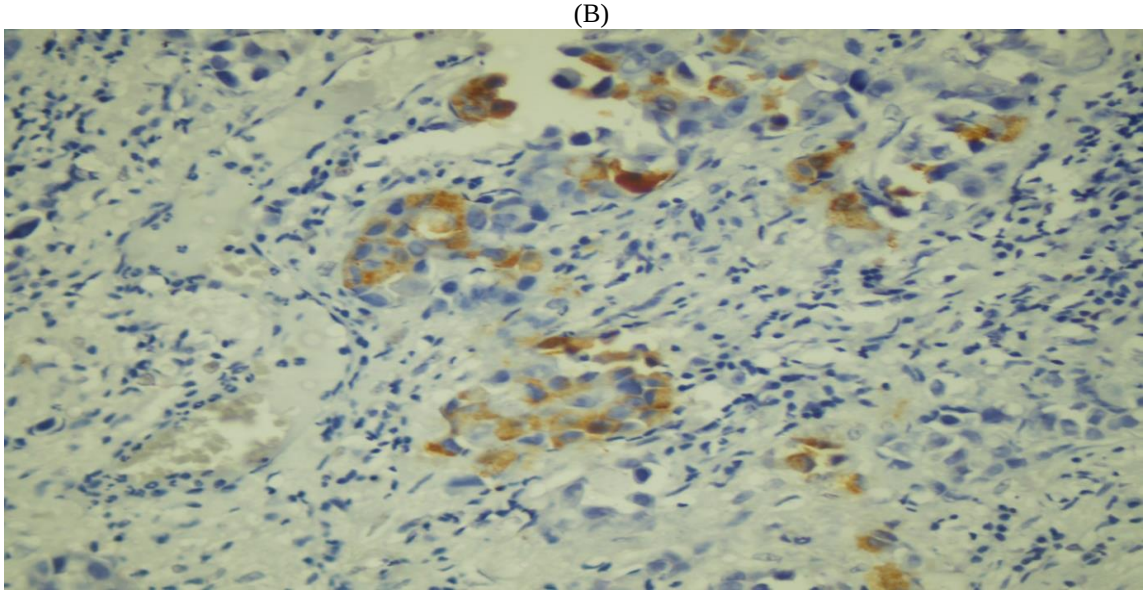
Patients' demographics

Out of one hundred seventy-six tumor tissue samples, only 132 had accessible medical records to collect data. The mean age was 59 years with majority of patients were 60 years or more (78.79%) and 98 cases (74.24%) diagnosed by TUR. Majority was non-metastatic bladder cancer but 76.52% diagnosed as late stage. More than one-third had primary tumor surgical resection (36.36%) while nearly two-thirds had radiotherapy and 60% had chemotherapy treatment. Transitional cell carcinoma (TCC) was the dominant pathological subtype in 95 samples (71.97%) while only 27 samples (20.45%) was squamous cell carcinoma (SCC) with 67.42% were high grade and 35.6% associated with bilharziasis. Larger tumor size (T3/T4) was presented in 94 cases (71.21%) and nodal tumor spread was present in 37.88%. About two-thirds (65.15%) tested positive for HER2 expression. [Table1; Fig. 1]



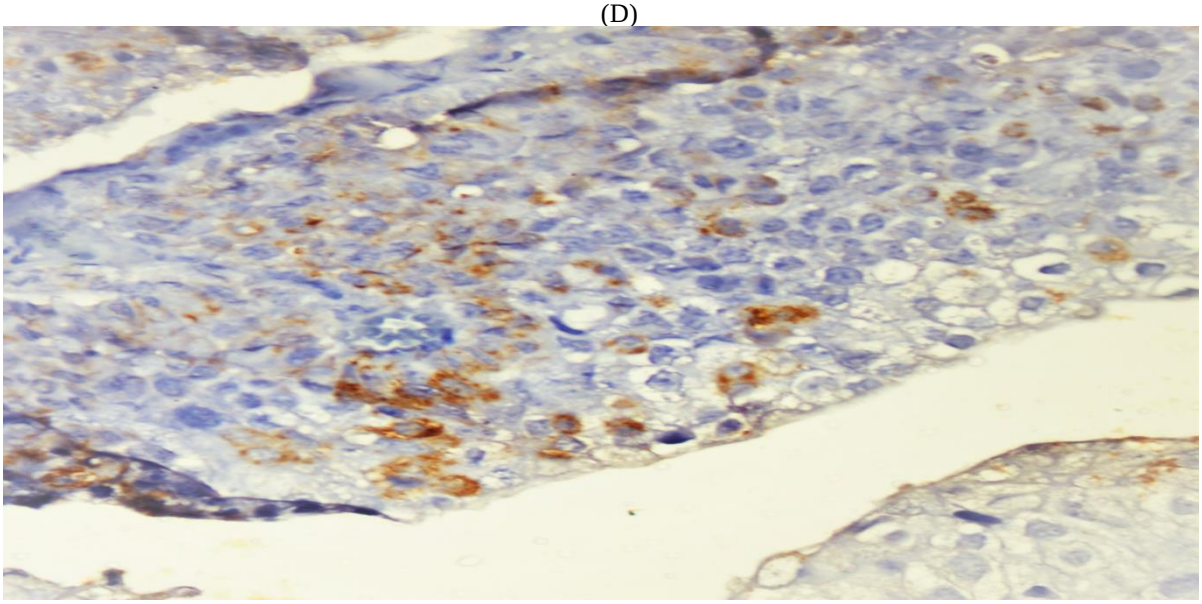
(A) Strong diffuse expression of HER2 in urothelial carcinoma cells; score 95% (X400)

(B)



(C) Moderate positive expression of HER2 in considerable number of urothelial carcinoma cells; score 40% (X400).

(D)



(C) Moderate positive expression of HER2 in sporadic cells of urothelial carcinoma cells; score 15% (X400).

Figure 1: IHC staining of the study group tissues. (A) Strong diffuse expression of HER2 in urothelial carcinoma cells; score 95% (X400). (B) Moderate positive expression of HER2 in considerable number of urothelial carcinoma cells; score 40% (X400). (C) Moderate positive expression of HER2 in sporadic cells of urothelial carcinoma cells; score 15% (X400).

Table 1: Patients' characteristics

Variable	Summary statistics
Age/year	
Mean ± SD	59.33±10.80
Age	
<60 years	28 (21.21%)
≥ 60 years	104 (78.79%)
Method of tissue diagnosis	
TUR	98 (74.24%)
Palliative cystectomy	1 (0.76%)
Partial cystectomy	2 (1.52%)
Radical	31 (23.48%)
Pathological type	
Adenocarcinoma	2 (1.52%)
Spindle cell tumor	1 (0.76%)
Squamous cell tumor	27 (20.45%)
TCC	95 (71.97%)
Undifferentiated carcinoma	7 (5.30%)
M	
M0	119 (90.15%)
M1	13 (9.85%)
N	
N0	82 (62.12%)
N1-N2	50 (37.88%)
T	
T1/ T2	38 (28.79%)
T3/ T4	94 (71.21%)
Clinical staging	
Early stage (I-II)	31 (23.48%)
Late stage (III-IV)	101 (76.52%)
Bilharziasis	
No	85 (64.39%)
Yes	47 (35.61%)
Grade	
Low grade (I-II)	43 (32.58%)
High grade (III)	89 (67.42%)
Surgery	
No	84 (63.64%)
Yes	48 (36.36%)
Radiotherapy	
No	46 (34.85%)
Yes	86 (65.15%)
Chemotherapy	
No	53 (40.15%)
Yes	79 (59.85%)
HER 2 intensity	
Negative	46 (34.85%)
Positive	86 (65.15%)

Patients' data and HER expression

HER2 expression was positive in 65% of samples; only pathological subtype showed significant association with HER expression intensity (p= 0.001) as TCC was the dominant pathology (83.7%) expression among HER2 positive samples while SCC pathology had only 12.79%. No significant association found between HER2 expression and other variables as regards age, muscle invasion, tumor size, nodal spread or stage. [Table2]

Survival

HER2 expression was associated with statistically significant longer cumulative overall survival (OS) with 18.7 months for HER2 positive and 9.7 months for HER2 negative patients (p=0.006). No statistically significant association found in the cumulative DFS and PFS when analyzed for HER2 expression status (p= 0.79 and p= 0.08 respectively). [Table 3]

Among other characteristics; older age and metastatic groups were associated with significantly shorter cumulative progression free survival (PFS) with 50.3 months for elderly (≥60) and 85.4 months for metastatic bladder cancer patients (p=0.04 and p= 0.02; respectively). Patients had primary tumor resection had statistically significant longer cumulative PFS with 94.7 months compared to 86.5 months in non surgical group (p=0.04). [Table 3]

In a multivariate Cox-regression analysis of factors affecting DFS, PFS and OS; HER2 expression was associated with statistically significant longer OS (p=0.04; HR=0.43). No statistically significant association between HER2 expression and DFS or PFS. Patients with higher ages had a significant shorter DFS (p=0.02; HR=2.8). [Table 4; Fig. 2-5]

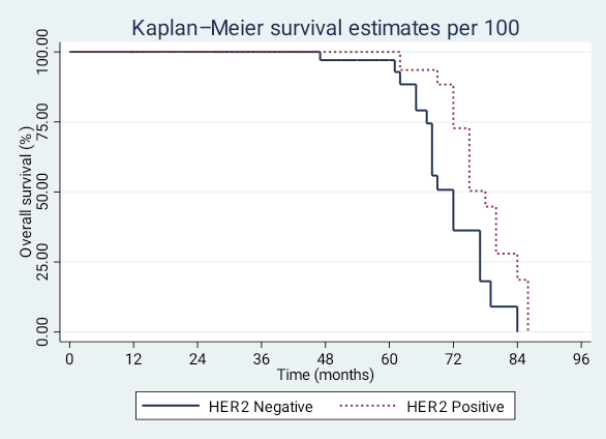


Figure 2: Relation between HER2 and overall survival

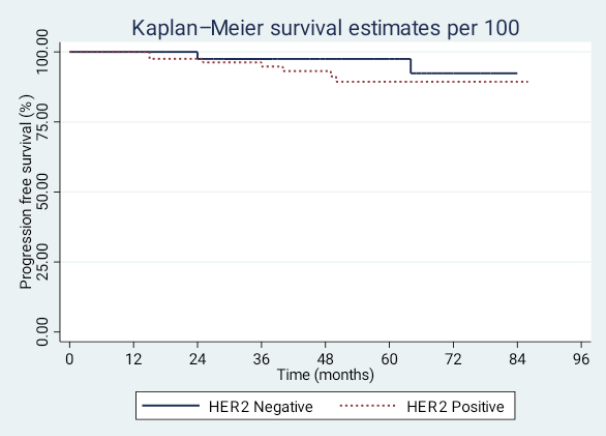


Figure 3: Relation between HER2 and PFS

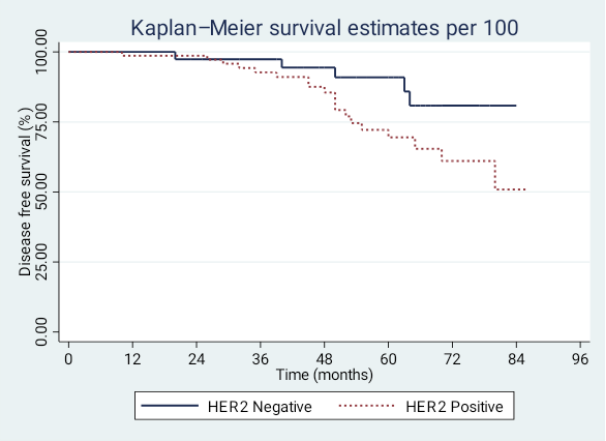


Figure 4: Relation between HER2 and DFS

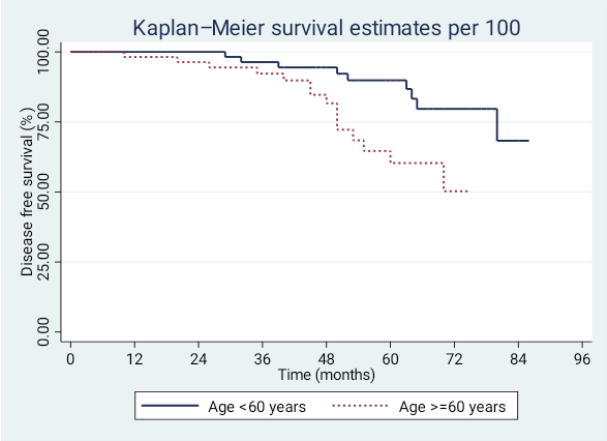


Figure 5: Relation between age and DFS

Table 2: Relation between HER2 intensity and other variables

Variable	HER2 intensity		P value
	Negative N=46	Positive N=86	
Age/year			
Mean ± SD	58.37±10.34	59.85±11.06	0.46
Age			
<60 years	7 (15.22%)	21 (24.42%)	0.22
≥ 60 years	39 (84.78%)	65 (75.58%)	
Pathological type			
Adenocarcinoma	2 (4.35%)	0	
Spindle cell tumor	1 (2.17%)	0	0.001
Squamous cell tumor	16 (34.78%)	11 (12.79%)	
TCC	23 (50.00%)	72 (83.72%)	
Undifferentiated carcinoma	4 (8.70%)	3 (3.49%)	
M			
M0	42 (91.30%)	77 (89.53%)	1.00
M1	4 (8.70%)	9 (10.47%)	
N			
N0	29 (63.04%)	53 (61.63%)	0.87
N1-N2	17 (36.96%)	33 (38.37%)	
T			
T1/ T2	11 (23.91%)	27 (31.40%)	0.37
T3/ T4	35 (76.09%)	59 (68.60%)	
Clinical staging			
Early stage (I-II)	9 (19.57%)	22 (25.58%)	0.44
Late stage (III-IV)	37 (80.43%)	64 (74.42%)	
Grade			
Low grade (I-II)	17 (36.96%)	26 (30.23%)	0.43
High grade (III)	29 (63.04%)	60 (69.77%)	

Table 3: Probability of DFS, PFS and overall survival at 60, 72 months and end of follow up according to different feature of studied populations

	DFS Cumulative survival%			P value	PFS Cumulative survival%			P value	OS Cumulative survival%			P value
	At 60 month	At 72 month	At last follow up (ms)		At 60 month	At 72 month	At last follow up (ms)		At 60 month	At 72 month	At last follow up (ms)	
All	77.5	67.6	57.9 (86ms)		92.2	89.9	89.9 (86ms)		98.8	55.0	9.7 (84 ms)	
Age/years												
<60	89.9	79.7	79.7 (75ms)	0.008	98.2	94.8	94.8 (75 ms)	0.04	98.0	54.0	50.2 (75 ms)	0.70
≥60	60.3	50.3	50.3 (75ms)		85.4	85.4	85.4 (75 ms)		100	46.9	46.9 (72 ms)	
Pathological type												
TCC	72.4	67.9	67.9 (78ms)	0.74	90.6	90.6	90.6 (78 ms)	0.68	100	55.3	27.7 (78 ms)	0.09
Squamous cell	89.6	-	63.9 (70ms)		94.4	-	85.0 (70 ms)		95.5	70.5	70.5 (73 ms)	
tumor	83.3	83.3	83.3 (79ms)		100	100	100 (79 ms)		100	-	26.7 (67 ms)	
Others												
M												
M0	77.5	67.6	57.9 (86ms)		-	-	95.3 (43 ms)	0.02	-	-	100 (43 ms)	1.00
M1	-	-	-		-	-	83.3 (43 ms)		-	-	100 (43 ms)	
Surgery												
No	72.1	60.7	60.7 (78ms)	0.08	86.5	86.5	86.5 (80 ms)	0.047	97.8	45.9	5.7 (84 ms)	0.08
Yes	83.7	73.9	73.9 (86ms)		100	94.7	94.7 (86 ms)		100	65.6	12.1 (84 ms)	
HER2												
Negative	90.9	80.8	80.8 (84ms)	0.08	97.5	92.4	92.4 (84 ms)	0.79	97.1	36.3	9.7 (79 ms)	0.006
Positive	96.5	61.1	50.9 (86ms)		89.4	89.4	89.4 (80 ms)		100	73.8	18.7 (84 ms)	

Table 4: Multivariate Cox regression analysis showing hazards ratio of factors affecting DFS, PFS and overall survival*

	HR (95% CI)	P value
	For DFS	
Age/years		
<60	Ref.	
≥60	2.81 (1.17:6.73)	0.02
Surgery		
No	Ref.	
Yes	0.50 (0.21:1.19)	0.12
HER2		
Negative	Ref.	
Positive	2.13 (0.79:5.79)	0.14
	For PFS	
Age/years		
<60	Ref.	
≥60	3.95 (0.79:19.79)	0.10
M stage		
M0	Ref.	
M1	4.25 (0.44:40.82)	0.21
Surgery		
No	Ref.	
Yes	0.19 (0.02:1.49)	0.11
	For OS	
Surgery		
No	Ref.	
Yes	0.49 (0.22:1.07)	0.08
HER2		
Negative	Ref.	
Positive	0.43 (0.20:0.95)	0.04

* (include only significant variable by log rank test)

Discussion:

Bladder cancer in Egypt has a higher prevalence than global rates. Also, Egypt ranked to have the top worldwide mortality from bladder cancer. [17] Chemotherapy despite being the systemic treatment of choice; is not a curative for advanced bladder cancer cases with increasing need for other therapies like targeted therapy or immunotherapy. [6, 7]

Variable rates of HER2 over-expression identified in bladder cancer [8https://www.spandidos-publications.com/10.3892/mco.2018.1786 - b75-mco-0-0-1786-12] with survival rates suggested to be clinically related. [9, 13]

In this work, analyzing 132 tumor samples of bladder cancer patients treated at our centers, the mean age was 59 years with majority of patients were 60 years or more. Majority was non-metastatic bladder cancer but 76.52% diagnosed as late stage. TCC was the dominant pathological subtype (71.97%) and 20.45% was SCC with 67.42% were high grade and 35.6% associated with bilharziasis. [Table1]

HER2 overexpression in bladder cancer reported to have a wide variable range from 31% and 65.5% [9-12] while in other series was from 2% to 71% in tested samples. [18] Lae M, et al reported that HER2 protein oevrexpression in bladder cancer ranges from 21% to 89%. [10]

Our data were in concordance with this range as in the current study, HER2 protein expression was positive in 65% of samples. The differences between the results reported may be due to different techniques and methods of assessment.

TCC was the dominant pathology and it showed the highest expression rate (83.7%) among HER2 positive samples while SCC pathology had only 12.79%. This discordance showed a statistically significant association between TCC pathology and HER expression intensity (p= 0.001). [Table2] This matched the results from Krüger S, et al. study. [19]

No significant association found between HER2 expression and other variables as regards age, muscle invasion, tumor size, nodal spread or stage. [Table2] This was in agreement with Lipponen P, et al work as there was no established correlation between HER2 protein and tumor stage or lymph node status. [19] Similar results reported by other researchers as HER2 over expression was not correlated with tumor stage, lymph node metastasis, grade or recurrence of the disease (P < 0.161). [10, 20-26]

In contrary; Krüger S., et al. reported that HER2 overexpression was significantly associated with higher

TCC grades (40%) and stages (38%). [27] Likewise, El ochi M.R. et al showed a significant correlation of HER2 overexpression with tumor grade ($p = 0.003$) and pathological stage ($p = 0.015$) but only tumor grade sustained that correlation in multivariate analysis ($P = 0.04$). [28] Similarly; high grade and stage correlated significantly with HER2 overexpression in other studies. [12, 29-31]

This study did not find significant association of HER2 and degree of bladder cancer invasiveness. This was similar to a result reported by Kumar S et al. as no statistical significance difference of HER2 expression in superficial or invasive bladder tumor ($P = 1.00$) was noticed. [20] However, these results were not in match with the results from other work. [32-36]

Prognosis and survival

Controversy about the value of HER2 expression and survival yet to be resolved; earlier studies did not show a possible prognostic value but others suggested that it was an independent variable in determining patient survival [37].

In the present work, HER2 expression showed appositive association with longer cumulative overall survival (OS) with 18.7 months for HER2 positive and 9.7 months for HER2 negative patients ($p=0.006$). In a multivariate Cox-regression analysis of factors affecting DFS, PFS and OS; HER2 expression was the only factor associated with statistically significant longer OS ($p=0.04$; HR=0.43). [Table 3, Table 4] [Fig. 1]

This was not in agreement with the results of Lipponen et al., work that linked increased HER-2 expression to aggressive bladder cancer and poor disease-specific survival [27, 35, 38]. Also, Zhao et al., reported same results of HER2 overexpression association with poor prognosis in UB cancer. [39] However, other reports matched our results and indicated no correlation with prognosis. [40, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4872193/ - bibr44-1756287216638981, 41]

Another study by Inoue et al., showed strong association between bladder cancer-specific survival with HER2 overexpression considering it as an independent risk factor for death at all time points. [42]

In this study, no statistically significant association found in the cumulative DFS and PFS when analyzed for HER2 expression status ($p= 0.79$ and $p= 0.08$ respectively). [Table 3]

Also, in multivariate Cox regression analysis, no significant association found between HER2 expression and DFS or PFS. [Table 4; Fig. 2; Fig. 3]

In contrary to our work, several studies suggested higher recurrence and progression rates if HER2 protein expression was high. [26, 32, 33, 35, 42-44]

A recent meta-analysis by Gan et al., found that HER2 protein overexpression was highly correlated with tumor recurrence, progression, and recurrence-free survival in patients with UB cancer. [45]

Because of these controversies, bladder cancer has limited Anti-HER2 options. However, a recent study reported a complete remission of the urothelial carcinoma following third-line treatment with Anti-HER2 plus chemotherapy in a patient with HER2 positive cancer after failure of frontline platinum-based combination chemotherapies. [46]

This may give our study a higher value if the evidence for using anti-HER2 grew to be widely accepted as it is the first work to provide an insight of HER2 expression among bladder cancer patients in our geographical region.

Among other characteristics; older patients had a significant shorter DFS in the multivariate-Cox analysis ($p=0.02$; HR=2.8). [Table 4; Fig. 4]

Limitations

The present work had some limitations. Only one method used to test for HER-2 protein detection. The current study used a control from another tumor samples (breast) instead of using different urothelial controls. Larger cohort of tumor sample is required for validation.

Conclusion:

Herein, we reported that 65% of our patients' cohort had Her2 over-expression.

Although requiring further validation, this expression showed a significant predictive value for overall survival. Also, TCC pathology significantly had a higher HER-2 expression. No significant association found among other variables in relation to HER2 expression. Additional studies required to validate these outcomes in parallel with investigating anti-HER2 treatment effect on these variables.

List of abbreviations

HER2= human epidermal growth factor receptor-2
UB= urinary bladder
DFS= disease free survival
PFS= progression free survival
OS= overall survival
TUR= transurethral resection
TCC= transitional cell carcinoma
SCC= sqaumous cell carcinoma

Conflict of interest

None declared.

Author's contributions

All authors carried out study design, data collection, analysis, interpretation of data, manuscript editing, the sequence alignment, and in the decision to submit the manuscript for publication. All authors read and approved the final manuscript. Dr. Ahmed RH carried out the pathological diagnosis and the immunohistochemistry technique, collecting results of both methods and scoring.

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