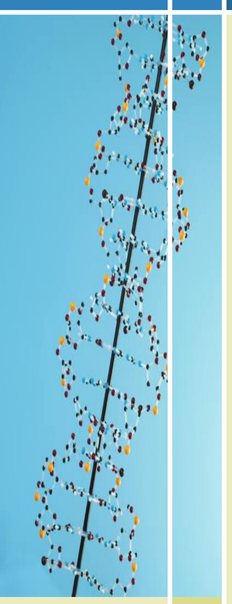
The prognostic role of EGFR and HER2 protein and gene expression in Urothelial Bladder Carcinoma

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Background

- Bladder cancer (BC) is the 5th most common malignancy in the Western world(1), and constitutes approximately 30% of all malignancies in Egypt, wherein bilharzial-associated BC represents the most common malignant tumor among male patients (2).
- Many authors validated the heterogeneity of BC, with different outcomes seen in patients at the same pathological stage or grade (3). Hence, it is crucial to determine both the prognostic and the predictive role of molecular markers in BC to identify potential therapeutic targets.
- Human epidermal growth factor (HER) family of receptor tyrosine kinase (RTK) is involved in normal bladder cell differentiation. It is composed of epidermal growth factor (EGFR/ErbB1/HER1), human EGFR 2 (ErbB2/HER2/NEU), as well as human EGFR3 (ErbB3/HER3) and human EGFR 4 (ErbB4/ HER4). Upon ligand binding and receptor dimerization, transmitted cytoplasmic signal activates a host of downstream signaling cascades, including PKC, PI3K, RAS, SRC, ABL, PAK and STAT5. The activated intracellular pathways enhance various oncogenic processes including proliferation, angiogenesis, cell survival, and increased metastatic potential (4,5).
- Despite the fact that EGFR and HER2 are well-established therapeutic targets in various cancers, their prognostic and predictive relevance in BC remain controversial.

Aim of Work

To evaluate the gene and protein expression of both EGFR and HER2 receptors and their correlation to the various prognostic parameters and survival of a cohort of Egyptian BC patients.

Materials and Methods

- Paraffin-embedded tissue samples from 46 patients with urothelial BC referred to Suez Canal University Teaching Hospital-Ismailia-Egypt between the years 2007 and 2014 were included in the study. In addition, 5 biopsy specimens from non-cancerous bladder tissue were used as controls.
- The follow-up data including treatment regimen, disease recurrence and /or progression, and survival were ascertained through the medical records of the patients retrieved from the Urology Department, Suez Canal University Teaching Hospital
- Immunostaining was performed using the primary antibodies: HER2/neu: Polyclonal rabbit antihuman c-erbB- 2 oncoprotein code No. A 0485 (Dako) at a dilution ratio of 1:250, and EGFR mouse monoclonal antibody (A-10): sc-373746 (Santa Cruz Biotechnology) at a dilution ratio of 1:200.according to manufacture's instructions.
- The EGFR and HER2 expressions were scored using the HercepTest criterion (figure 1,2)
- RNA was extracted from FPPE sections and gene expression was assessed, in relation to GAPDH ,using real time PCR.

Results and Discussion

- Overall, positive staining for EGFR and HER2 was detected in 35 % and 46% of BC patients, with 17.4% tumors exhibiting co-expression of both receptor proteins compared to 37% tumors negative for both proteins.
- As shown in table 1, EGFR protein expression increased significantly in large and high-grade advanced stage BC tumors (p value 0.027, 0.015 and 0.01 respectively). In contrast to previous reports (3,6), there was no significant association of HER2 expression with any of the studied clinico-pathologic parameters.
- A key valuable finding of our study is the significant simultaneous association of each of the studied RTK receptors with two prognostic factors, cancer recurrence and progression (table 1)

Table 1. Association of HER2 and EGFR protein expression with the clincopathologic characteristics of the studied urothelial carcinomas

Feature	No of cases (%)	EGFR protein expression		P value	HER-2 protein expression		P value
		-ve 30(65.2)	+ve 16(34.8)		-ve 25(54.3%)	+ve 21(45.7%)	
Gender				0.114*			1*
Male	42(91.3)	29(69)	13(31)		23(54.8)	19(45.2)	
Female	4(8.7)	1(25)	3(75)		2(50)	2(50)	
Age				0.536			0.139
≤60	23 (50)	14 (60.9)	9 (39.1)		10 (43.5)	13 (56.5)	
>60	23 (50)	16 (69.6)	7 (30.4)		15 (65.2)	8 (34.8)	
Size				0.027*			0.244*
<3cm	20(43.5)	17(85)	3(15)		13(65)	7(35)	
≥3 cm	26(56.5)	13(50)	13(50)		12(46.2)	14(53.8)	
Multiplicity				0.229*			0.372*
Single	26(56.5)	19(73.1%)	7(26.9)		16(61.5)	10(38.5)	
Multicentric	20(43.5)	11(55)	9(45)		9(45)	11(55)	
Bilharzia				1*			1*
Absent	33 (71.7)	21(63.6)	12(36.4)		18(54.5)	15(45.5)	
Present	13 (28.3)	9(69.2)	4(30.8)		7(53.8)	6(46.2)	
Grade				0.015*			0.136*
Low	26(56.5)	21(80.8)	5(19.2)		17(65.4)	9(34.6)	
High	20(43.5)	9(45)	11(55)		8 (40)	12(60)	
pT				0.01**			0.071**
Ta	4(8.7)	4(100)	0(0)		4(100)	0(0)	
T1	20(43.5)	17(85)	3(15)		13(65)	7(35)	
T2	11(23.9)	6(54.5)	5(45.5)		3(27.3)	8(72.7)	
T3	8(17.4)	2(25)	6(75)		3(37.5)	5(62.5)	
T4	3(6.5)	1(33.3)	2(66.7)		2(66.7)	1(33.3)	
Lymph node metastasis(n=24)				1*			0.423*
Negative	13(28.3)	6(46.2)	7(53.8)		6(46.2)	7(53.8)	
Positive	11(23.9)	5(45.5%)	6(54.5)		3(27.3)	8(72.7)	
Stage(n=24)				0.685**			0.791**
I	3(6.5)	2(66.7)	1(33.3)		1(33.3)	2(66.7)	
II	7(15.2)	4(57.1)	3(42.9)		3(42.9)	4(57.1)	
III	8(17.4)	3(37.5)	5(62.5)		2(25)	6(75)	
IV	6(13)	2(33.3)	4(66.7)		3(50)	3(50)	
Lymphovascular invasion				0.126*			0.235*
Negative	25(54.3)	19(76)	6(24)		16(64)	9(36)	
Positive	21(45.7)	11(52.4)	10(47.6)		9(42.9)	12(57.1)	
Recurrence				0.026			0.022
Absent	30(65.2)	23 (76.7)	7 (23.3)		20 (66.7)	10 (33.3)	
Present	16(34.8)	7 (43.8)	9 (56.3)		5 (31.3)	11 (68.8)	
Progression				0.026*			0.125*
Absent	30(65.2)	23 (76.7)	7 (23.3)		19 (63.3)	11(36.7)	
Present	16(34.8)	7 (43.7)	9 (56.3)		6 (37.5)	10 (62.5)	
Metastasis				0.602*			0.318*
Absent	42(91.3)	28 (66.7)	14 (33.3)		24 (57.1)	18(42.9)	
Present	4 (8.7)	2 (50)	2 (50)		1 (25)	3 (75)	

* Fisher's Exact test. ** Chi Square test.

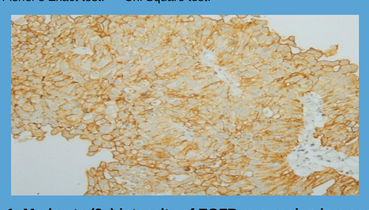


Figure 1: Moderate (2+) intensity of EGFR expression in a case of low grade urothelial carcinoma. (Hematoxylin & DAB, X 400).

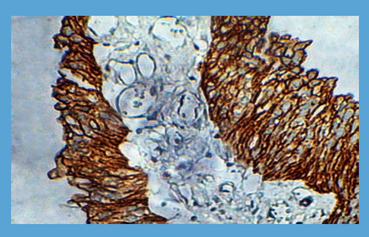


Figure 2: Marked (3+) intensity of HER2 expression in a case of high grade urothelial carcinoma. (Hematoxylin & DAB, X 400).

Table 2. Correlation of level of *HER2* & *EGFR* gene expression with the various clinicopathologic characteristics of the studied urothelial carcinomas

		EGFR	*HER2*
Grade	Correlation Coefficient	.080	-.254
	P value	.670	.134
Bilharzia	Correlation Coefficient	.027	.267
	P value	.884	.116
Stage	Correlation Coefficient	.069	-.294
	P value	.806	.209
pT	Correlation Coefficient	.170	-.254
	P value	.361	.135
Size	Correlation Coefficient	.274	-.099
	P value	.135	.565
Multiplicity	Correlation Coefficient	.246	-.111
	P value	.181	.519
Lymph node	Correlation Coefficient	-.252	-.270
	P value	.365	.249
L V invasion	Correlation Coefficient	.130	-.238
	P value	.484	.162
Recurrence	Correlation Coefficient	-.113	-.276
	P value	.545	.104
Progression	Correlation Coefficient	-.087	-.268
	P value	.642	.113
Metastasis	Correlation Coefficient	.037	.023
	P value	.845	.892

Figure 3. Kaplan Meier survival for patients with and without EGFR protein expression and disease free survival (DFS)

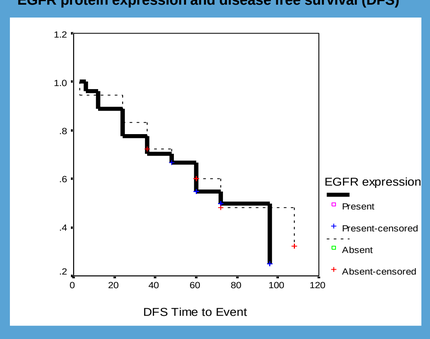
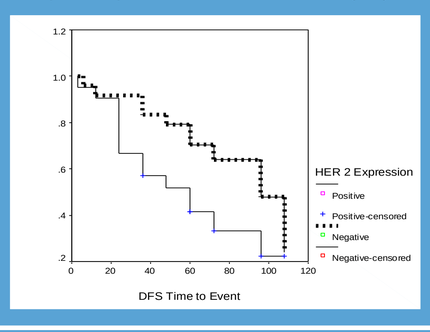


Figure 2. Kaplan Meier survival for patients with and without HER2 protein expression and disease free survival (DFS)



- Gene expression was significantly higher in tumor samples in comparison to the controls (10.6 and 21 folds for EGFR and HER2).
- There was no evidence that either EGFR or HER2 mRNA expression is linked to BC grade, stage, tumor size, multiplicity or lymph node metastasis (data not shown). Likewise, Spearman analysis revealed lack of correlation between EGFR or HER2 mRNA levels with any of the prognostic variables (table 2).
- The present study proposed a significant EGFR protein-mRNA concordance, but a modest protein-mRNA relation for HER2. Other authors have reported similar findings (7,8).
- Inconsistent with other researches (8,9), our data did not provide compelling evidence that EGFR protein and mRNA expression are significantly implicated in the urothelial transformation of bilharzial BC.
- Interestingly, HER2 mRNA exhibited 3-fold increase in Bilharzia-positive tumors compared to the negative ones.
- The mean follow- up period for the current cohort was 78.27± 18.487 months (median 72, range 36-108 months)
- Of note, univariate Kaplan-Meier survival analysis verified HER-2 overexpression as a predictor of DFS with borderline significance (figure 4).
- Moreover, multivariate analysis revealed that only lymph node metastasis was a significant independent predictor of OS of the studied cohort, while tumor multiplicity and distant metastasis were of borderline significance. These findings are in partial agreement with previous studies (3, 10-12).

Conclusion

The study reported aberrant expression of both EGFR and HER2 in BC and elaborated that EGFR protein overexpression was associated with high tumor grade and advanced stage. Additionally, significant co-expression of both proteins was predominantly detected in muscle-invasive tumors. Although patients with tumors overexpressing both receptor proteins experienced a significantly higher recurrence rate, only HER2 overexpression influenced the disease-specific survival. Until detailed molecular evaluation of the various interactive partners for each HER-RTK member in BC is revealed, Our data highlight the prognostic significance of EGFR and HER in BC and propose their use as predictive markers as well as therapeutic targets.

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References

1-Jahnson, S., et al., *Swedish National Registry of Urinary Bladder Cancer: No difference in relative survival over time despite more aggressive treatment.* Scand J Urol, 2016. 50(1): p. 14-20.
2-Eissa, S., et al., *Urine biomarkers of schistosomiais and its associated bladder cancer.* Expert Rev Anti Infect Ther, 2015. 13(8): p. 985-93.
3-Zhao, J., et al., *Prognostic role of HER2 expression in bladder cancer: a systematic review and meta-analysis.* Int Urol Nephrol, 2015. 47(1): p. 87-94.
4-Ullrich, A. and J. Schlessinger, *Signal transduction by receptors with tyrosine kinase activity.* Cell, 1990. 61(2): p. 203-12.
5-Arteaga, C.L. and J.A. Engelman, *ERBB receptors: from oncogene discovery to basic science to mechanism-based cancer therapeutics.* Cancer Cell, 2014. 25(3): p. 282-303.
6-El Ochi, M.R., et al., *Expression of human epidermal growth factor receptor 2 in bladder urothelial carcinoma.* BMC Clin Pathol, 2017. 17: p. 3.
7-Bellmunt, J., et al., *HER2 as a target in invasive urothelial carcinoma.* Cancer Med, 2015. 4(6): p. 844-52.
8-Khaled, H.M., et al., *Clinical significance of altered nm23-H1, EGFR, RB and p53 expression in bilharzial bladder cancer.* BMC Cancer, 2009. 9: p. 32.
9-Badawy, A.A., et al., *Expression of Epidermal Growth Factor Receptor and Transforming Growth Factor Alpha in Cancer Bladder: Schistosomal and Non-Schistosomal.* Curr Urol, 2017. 9(4): p. 192-201.
10-Nedjadi, T., et al., *Prognostic value of HER2 status in bladder transitional cell carcinoma revealed by both IHC and BDISH techniques.* BMC Cancer, 2016. 16: p. 653.
11-Chen, P.C., et al., *Her2 amplification distinguishes a subset of non-muscle-invasive bladder cancers with a high risk of progression.* J Clin Pathol, 2013. 66(2): p. 113-9.
12-Kruger, S., et al., *HER2 overexpression in muscle-invasive urothelial carcinoma of the bladder: prognostic implications.* Int J Cancer, 2002. 102(5): p. 514-8.