EUROPEAN ACADEMIC RESEARCH
Vol. III, Issue 11/ February 2016

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

HER-2/Neu over-expression in invasive Bladder Cancer in Sudanese Patients

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Abstract:

***Background**: Bladder cancer is one of the commonest malignancies with high morbidity and mortality rates. Human Epidermal Growth Factor Receptor -2 (Her-2/Neu) seems to play role in the pathogenesis and prognosis of bladder carcinomas; However it is expression and prognostic significance were variable between different studies.*

***Objective:** The purpose of this study was to determine if any relationship exists between expression of Her2/Neu marker and invasive urinary bladder cancers.*

***Method:** Tissue samples were collected from 35 patients with invasive bladder lesions, 26 male 9 female were analyzed for HER-2 over-expression using immunohistochemistry during the period from August to December 2015.*

***Result:** HER2/Neu was found to be insignificantly overexpressed in the histopathological types of invasive bladder samples (p=0.55) and no significant correlation between HER-2 over-expression and grade of bladder carcinoma (p=0.06).*

Conclusion: *This study found there is no correlation between HER-2 over-expression and invasive bladder cancer.*

Key words: Her-2/Neu, over-expression, immunohistochemistry, bladder cancer

INTRODUCTION:

Bladder cancer considers a significant health problem, as it is the one of the most common cancers (1). Worldwide, bladder cancer is diagnosed in approximately 275,000 people each year, and about 108,000 die of this disease (2).

Bladder cancer is three to four times more common in men than in women, age-standardized (world) mortality rates are 2-10 per 100,000 males and 0.5-4 per 100,000 females(3).

This is strikingly higher than most other parts of the world. Despite the progress of therapeutic means, up to 30% of patients who suffer from noninvasive tumors in the muscularis propria and 50% of those with invasive tumors in the detrusor muscle present with recurrences or they die (4, 5).

The human epidermal growth factor receptor-2 (Her-2/Neu, also known as erbB2 and Her-2), encoded by c-erbB2 gene localized to chromosome 17q, is a 185 kDa membrane-spanning glycoprotein with tyrosine kinase activity. Ligand binding of these receptors causes its dimerizations and activates the intracellular kinase domain, leading to phosphorylation of tyrosine residues and the transduction of mitogenic signals. Thus, Her-2/Neu plays a fundamental role in cell growth, differentiation survival, and migration (6), and abnormal activation of Her-2/Neu leads to oncogenic transformation of the cell (7).

Her-2/Neu gene amplification and/or the protein overexpression have been long time considered as bad prognostic factors in several carcinomas, particularly in breast and ovarian carcinomas (8, 9). Recently, anti-Her-2/Neu

antibody, also known as transtuzumab or Herceptin, has shown its efficiency in these tumors' therapy, both alone and in combination with Cisplatin. Consequently, assessment of Her-2/Neu status has assumed therapeutic significance (10).

Her-2/Neu appears to play role in the pathogenesis of bladder carcinoma; however, its expression was variable between different studies, ranging between 9 and 81% (11, 12). Furthermore, analysis of Her-2/Neu overexpression has been the subject of numerous studies in an attempt to make correlations with clinical pathological parameters of prognosis, with discrepant results (3, 13,14). It has been identified as an independent predictor for disease-related survival in muscle-invasive bladder carcinomas (15).

PATIENTS AND METHOD:

Thirty-five invasive urinary bladder carcinomas were examined in this study. Only invasive bladder tumors were included based on availability of representative paraffin embedded tumor tissue, 26 of this patients were male and 9 were female. Tissue processed was performed by histological technique and reviewed histological subtype, grading following the World Health Organization (WHO) classification. Paraffin-embedded tumor specimens were sectioned at 4- 5 μm on coated slides and subjected to IHC , sections were heated in citrate buffer (ph. 6.0 at 95 ˚C) and immunostained using polyclonal rabbit anti-substrate for visualization ,and final Mayer's hematoxylin for counterstaining .

Scoring of HER-2 over-expression:

Immunostained sections were evaluated by pathologist under microscope. Scoring for membranous staining was done using the following system: 1+ at most faint, equivocal, and incomplete membranous staining; 2+, unequivocal, complete

membranous pattern, with moderate intensity ; and 3+, strong, membranous pattern . Score of 2 or 3 were considered positive for Her-2(10).

STATISTICAL ANALYSIS:

Statistical analysis performed by SPSS version 22, Pearson's Chi-square test assessed the correlation between Her-2 protein expression and other invasive bladder carcinoma variables , statistical significant of P-values were considered when $P<0.05$ was reached.

RESULT:

The histological subtype of the 35 bladder carcinomas, transitional cell carcinoma was 26 (74.3%) and squamous cell carcinoma was 6 (25.7%). The most grading is grade 1 and grade 3 (48.6%, 42.9% respectively). For immunostain, 27 (77.1%) were negative (staining intensity 1+), whereas 8 (22.8%) were positive (staining intensity 2+ or 3+ in 6 and 2 patients, respectively).There is no significant correlation between Her-2/Neu protein over-expression and histopathologic type (TCC, SCC) (P=0.55) (Table.1).as well as, there is no significant association between Her-2/Neu over-expression and tumor grade (P=0.06) (Table.2).

Table (1): Frequency of HER-2/Neu expression according to histological type of invasive bladder cancer

Histological type		HER			Total
		Strong positive	Weak positive	Negative	
Diagnosis	transitional cell carcinoma	2	5	19	26
	squamous cell carcinoma	0	1	8	9
Total		2	6	27	35

P- Value=0.55

Table (2): The relation between HER-2/Neu expression and grade of bladder carcinoma

Grade		HER			Total
		Strong positive	Weak positive	Negative	
Grade	grade 1	1	5	11	17
	Grade 2	1	0	2	3
	Grade 3	0	1	14	15
Total		2	6	27	35

P-Value=0.06

DISCUSSION:

Our study found there was no relation between her-2/Neu expression and histopathological type of bladder cancers (P=0.55) in agreement with some studies (16, 17, 18) have found no such prognostic significance.

Previous studies on HER-2 expression in bladder carcinoma using Western blot analysis and IHC have found a correlation between increased HER-2 expression and both higher tumor stage and grade (20,21).but our study contradicted these studies, we found no significant correlation between HER-2 over-expression and histological subtype of bladder cancer (TCC, SCC) (p=0.55), and also no significant correlation between HER-2 over expression and tumors grade (P=0.06). Others (16, 19, 22) have reported a significant association between HER-2 over-expression and a higher tumor grade. Thus, shawky et al. (23) studied HER-2 over-expression in invasive bladder carcinoma among cohort patients. HER-2 over-expression was associated with high-grade bladder cancers. Similarly, in a recent study (24) on 59 patients, HER-2 over-expression was significantly correlated with the differentiation grade, our study contradicted for these studies; we found no significant association between HER-2/Neu expression and the grade of bladder carcinoma(P=0.06).

In some studies (25, 26) there was no significant association between HER-2 and either pathological staging or tumor grading. Similarly, in a series of 53 bladder cancers, no significant correlation was found between HER-2 and stage or

grade (27). Furthermore, in that study, the authors reported no significant difference between TCC and SCC groups, our study confirmed this finding, we found no significant correlation between HER-2/Neu expression and tumor grade.

In El Gehani et al (28), it seems likely that the reported variations in HER-2 expression in urothelial cancer may be the result not only of true biological variations but also of several confounding variables in these studies. These include the use of different antibodies for IHC, different criteria for IHC-positivity (i.e. cytoplasmic or membrane staining), and different scoring criteria.

Differences in both the incidence of Her-2/Neu expression and its prognostic significance in bladder carcinoma could be explained by different methodologies (gene amplification versus protein overexpression), or different techniques used (PCR, fluorescence in situ hybridization, and immunohistochemistry), and different antibodies applied for IHC, in addition to the inconsistent criteria set for detecting IHC positivity(23).

Despite the inconclusive data on the prognostic value of HER-2 as an independent marker of tumor progression, there may be a therapeutic role for an anti HER-2 agent such as trastuzumab in cancer treatment (28). Available data clearly demonstrate that the development of new drugs will have little, if any, chance of success if it is not guided by in-depth knowledge of disease biology. However, using biologic agents to target key molecular pathways, such as those regulated by HER family members, may be effective. Indeed, the positive results achieved by trastuzumab in breast and gastric HER-2 positive tumors support this approach (28-29). Combining trastuzumab with chemotherapy in HER-2 positive advanced gastric cancer was significantly more effective than chemotherapy alone (30) and has a favorable toxicity profile (31).

CONCLUSION:

Our study showed that there was no significant correlation between HER-2/Neu expression and histological subtype (TCC, SCC) of bladder carcinoma (p=0.55), as well as there was no significant association between expression of HER-2/Neu and grade of urinary bladder cancer (p=0.06).

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