Overexpression of HER-2\neu receptor protein in Urinary Bladder Carcinoma, An Immunohistochemical Study.

Rawaa G Al-Tereihi* MBChB, M.Sc
Hadeel A..Kerbel** MBChB, FICMS (Path.)
Rehab H. Saheb** MBChB, FICMS (Path.)
As'ad A. Al-Janabi** MSc, PhD (Path.)
Akeel A. Yasseen** MSc (UK), PhD (UK)

Summary:

Background: Bladder carcinoma is one of the most common cancer worldwide, it accounts for 6.5% of all cancers, with highest incidence in industrialized countries .It represents the fourth most common cancer in men and the eighth in women. Bladder carcinoma depends in its pathogenesis on a combination of genetic and environmental factors, these factors produce phenotypic changes that allow normal transitional cells to become cancerous and finally acquire the "malignant phenotype". Many attempts had been tried to explore the role of some genetic abnormalities encountered in bladder carcinoma. It has indicated that many genetic abnormalities may underline the pathogenesis of cancer evolution of urinary bladder like VEGF , P53, Bcl2 and RB, but nowadays a scientific efforts have raised the possible role of Her-2/neu in bladder carcinoma, and the rate of its overexpression in bladder carcinoma is ranging from 2% - 74%.

Objective: To estimate immunohistochemical expression of HER-2\neu receptor protein in Urinary Bladder Carcinoma in relation to other parameters; sex grade, pattern of tumor and stage of tumor .

Methods: The present work is performed in the Department of Pathology and Forensic medicine, College of Medicine, Kufa University. Formalin fixed, Paraffin-embedded blocks from 60 (43 males and 17 females) patients with urinary bladder carcinoma were included in this study. A group of 12 patients with chronic cystitis were included as a control group. Avidin-Biotin Complex method was employed for immunohistochemical detection of HER-2\neu.

Results: HER-2\neu overexpression was positive in 41.6% of urinary bladder carcinoma, while there was no expression in benign bladder tissue ($P<0.05$). HER-2\neu immunohistochemical staining was positively correlated with, grade and stage of urinary bladder carcinoma ($P<0.05$).

Conclusion: These findings support the role of HER-2\neu in the carcinogenesis of urinary bladder carcinoma regarding behavior, and aggressiveness, and thus HER-2\neu could be considered as a poor prognostic parameter in urinary bladder carcinoma.

Keywords: Gefr:gastresophageal reflex disease. Barrts esophagus, columnar lined lower esophagus.

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Introduction:

Bladder carcinoma is one of the most common cancers worldwide, it accounts for 6.5% of all cancers, with highest incidence in industrialized countries .It represents the fourth most common cancer in men and the eighth in women (1). Bladder carcinoma depends in its pathogenesis on a combination of genetic and environmental factors, these factors produce phenotypic changes that allow normal transitional cells to become cancerous and finally acquire the "malignant phenotype" (2, 3). Bladder carcinoma is predominantly a disease of the elderly male. The peak age of incidence is the seventh decade, and the male-to-female ratio is 3 to 1 (4). In Iraq, bladder carcinoma is recorded as the second most common carcinoma in males, and the ninth in females according to Iraqi cancer registry (5). In the United States, bladder carcinoma is the fourth most common type of carcinoma in men and the ninth most common carcinoma in women (7). Exposure to environmental carcinogens of various types is responsible for the development of most bladder carcinoma and the most important risk factors are: Cigarette smoking (8), Occupational Exposure like The occupational group of domestic helpers (9), Schistosomiasis (10), Chronic Cystitis (11), Drugs like Cyclophosphamide (13) & Radiation Therapy for ovarian (14), cervical (15), and prostatic carcinomas (16). Hereditary Factors i.e. familial clustering of bladder carcinoma, especially of relatively young individuals, has provided support to the concept that there may be a genetic component involved in some bladder carcinoma (17). The human epidermal growth factor receptor 2 (HER-2)/*neu* (c-*erbB*-2) gene is localized to chromosome 17q21 and encodes a transmembrane tyrosine kinase receptor protein that is a member of the epidermal growth factor receptor (EGFR) or HER family (18). This gene encodes 185,000 transmembrane phosphoglycoprotein , molecular weight 6045 Dalton protein with 53 amino acid , which are expressed in normal urothelium .This receptor possesses intrinsic tyrosine kinase activity and all members of this family are frequently overexpressed and \or their respective genes are amplified in human neoplastic tissue. HER-2\neu receptor has an important role in the regulation of

* *Dept. of Pathology, Faculty of Medicine, Babylon University.*
** *Dept. of Pathology, Faculty of Medicine, Kufa University.*

cell growth, proliferation and differentiation . Its overexpression has been associated with high grade and advanced stage of bladder carcinoma specially TCC (19).

Materials & methods:

Sixty formalin fixed, paraffin embedded samples (43 males & 17 females) from patients with urinary bladder carcinoma were collected from the major hospitals & private laboratories in Middle Euphrates region of Iraq. All biopsies were classified according to the modified world health organization classification, into three grades: grade I, grade II& grade III. The mean age of patients was 59.5 years. A group of 12 patients with benign bladder lesion (chronic cystitis) was used as a control population. Tissue sections of 5-Mm thickness from formalin-fixed, paraffin-embedded blocks were taken for immunohistochemistry. The avidin-biotin complex method was used for immunohistochemical detection of HER-2/neu. The criterion for positive reaction was a dark brown staining of the membrane. The scoring percentage were assessed by counting the percentage of positive cells in 100 malignant cells at 40x total magnification for at least 5 fields, four scaled scorings system were chosen in this study (20):

Score 0 completely negative.

Score +1 faint perceptible staining of the membrane (< 10% of malignant cells).

Score +2 moderate staining of the partial membrane in > 10 % of the tumor cells.

Score +3 strong circumferential staining of the entire membrane

creating a fishnet pattern (in > 10% of malignant cells).

Positive staining for HER -2\neu should be considered if:

1- 67 %of the tumor cells should be stained.

2- The staining intensity should be moderate to intense (+2, +3).

3-The staining pattern should be membranous, with or with no concomitant cytoplasmic staining. The results were statistically evaluated by the help of SSPS version 10 software using Chi-square test and Correlation-Regression test.

Results:

In all sections of benign control bladder lesions (cystitis), none of them revealed a positive overexpression for ***HER-2\neu*** in the cell membrane. While in study group, ***HER-2\neu*** overexpression was reported in 25(41.66%) out of 60 cases of bladder carcinoma with highly significant difference in comparison with control group ($P < 0.0\ 5$) (Table.1) Fig 1& 2. There was a significant difference between the types of bladder carcinoma in relation to HER -2\neu protein ($P< 0.05$) (Table.1). Immunohistochemical analysis of HER-2\neu protein overexpression in relation to grade of tumor revealed that positive HER-2\neu was reported in 35% cases out of grade I, 38.88% grade II, and 50.00%o grade III, with a positive correlation between the detection rate of HER-2\neu immunohistochemical staining and grade of tumor, i.e., as tumor grade increase more HER-2\neu overexpression was noticed ($P < 0.05$) (Table. 1). HER-2\neu overexpression was not reported in any case of Ta, while Her-2\neu over expression was positive in 33.33% of T1, 44.44%of T2, 50% ofT3, and 58.82% of T4**.** There was a significant difference between superficial low grade tumor (Ta , T1) and the invasive high grade tumor (T2 ,T3 and T4) ($P < 0.05$) (Table.1). Regarding the scoring percentage of Her-2/neu, the correlation between the intensity & the grade of the tumor is shown in table.2 and there is a significant difference between the intensity of HER-2\neu and the grade of tumor ($P < 0.05$) ($R= 0.0192$). As summarized in Table.1, HER-2/neu overexpression in 40% of the samples from primary bladder carcinoma patients & 42.85% of those with recurrent carcinoma. This finding was not statistically significant ($p>0.05$). The same statistical result ($p>0.05$) was applied to the correlation between primary & recurrent tumors with the scoring percentage of HER-2/neu scoring. (Table.2) The scoring percentage of HER-2/neu overexpression in correlation to the stage of the tumor is summarized in Table.2, which detect no significant difference between the tumor stage & intensity of scoring. ($p>0.05$).

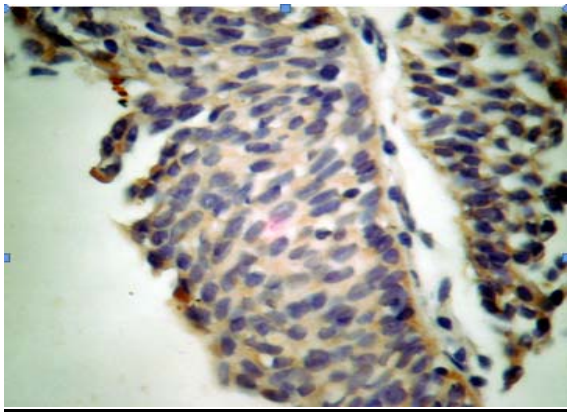

Figure.1: papillary transitional cell carcinoma grade I showing positive HER-2\neu receptor (membranous & cytoplasmic) immunoassaying score 1 (40X) .

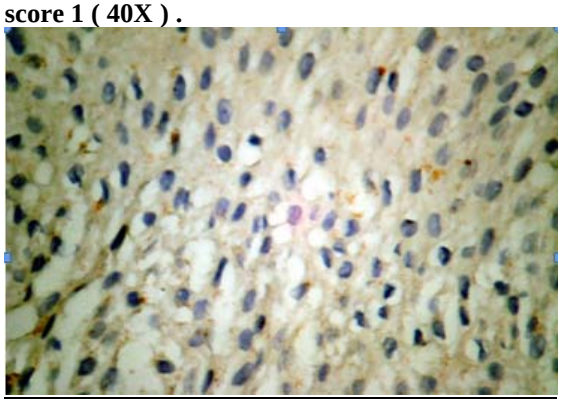

Figure2: Transitional cell carcinoma grade II showing positive HER-2\neu receptor of score 2(40x).

Table 1: Her2/neu overexpression in relation to type of tissue and cancer, grade, stage and recurrence of bladder cancer patients.

parameter	positive	negative	total	P value	R test
Type of tissue					
Benign (cystitis)	0	12(100%)	12(16.7%)	P<0.05	
Malignant (TCC)	25(41.66%)	35(58.33%)	60(83.3%)		
Type of cancer					
TCC	23(41.07%)	33(58.92%)	56(93.3%)	P<0.05	
SCC	1(33.33%)	2(66.67%)	3(5%)		
Adeno CA	1(100%)	0	1(1.7%)		
Grade					
Grade I	7(35%)	13(65%)	20(33.3%)	P<0.05	R=0.019
Grade II	7(38.88%)	11(61.11%)	18(30.0%)		
Grade III	11(50.00%)	11(50.00%)	22(36.7%)		
Stage (T)					
T a	0	8(100%)	8(13.33%)	P<0.05	
T1	4(33.33%)	8(66.67%)	12(20.00%)		
T 2	4(44.44%)	5(55.56%)	9(15.00%)		
T 3	7(50.00%)	7(50.00%)	14(23.33%)		
T 4	10(58.82%)	7(41.17%)	17(28.23%)		
Recurrence					
Primary	10(40%)	15(60%)	25(41.66%)	p>0.05	
Recurrent	15(42.85%)	20(27.14%)	35(58.33%)		

Table 2: The percentage score of Her2/neu overexpression in relation to grade stage, recurrence and pattern of immunostaining in TCC.

parameter	0	+1	+2	+3	total
Grade					
Grade I	10(50%)	3(15%)	3(15%)	4(20%)	20(33.3%)
Grade II	6(33.33%)	5(27.77%)	2(11.11%)	5(27.77%)	18(30%)
Grade III	4(18.18%)	7(31.81%)	3(13.63%)	8(36.36%)	22(36.7%)
					P< 0.05
Stage (T)					
T a	8(100%)	0	0	0	8(13.33%)
T 1	8(66.66%)	0	1(8.33%)	3(25%)	12(20%)
T 2	1(11.11%)	4(44.44%)	2(22.22%)	2(22.22%)	9(15%)
T 3	2(14.28%)	5(35.7%)	2(14.28%)	5(35.7%)	14(23.33%)
T 4	1(5.88%)	6(35.29%)	3(17.64%)	7(41.17%)	17(28.33%)
					P> 0.05
Recurrence					
Primary	12(48%)	3(12%)	2(8%)	8(32%)	25(41.66%)
Secondary	8(22.85%)	12(34.28%)	6(17.14%)	9(25.71%)	35(58.4%)
					p> 0.05

Discussion:

Human epidermal growth factor receptors are involved in signal transduction pathways resulting in proliferation , cell survival , angiogenesis , and metastasis (21) . ***HER-2/neu*** expression in bladder carcinoma is variable, ranging from 2%- 74% (3), These variations in reported rates of HER-2\neu expression are due to differences in the applied scoring criteria for the assessment of HER-2\neu overexpression (22).or could be attributed to tumor heterogeneity, sampling, and methods used. Several groups have reported heterogeneous Her-2/*neu* expression within tumors and observed differences in overexpression between primary tumors compared with metastases (23, 24) and they found a correlation between ***HER-2/neu*** overexpression with a more aggressive clinical course. Bladder carcinoma has a high rate of ***HER-2/neu*** overexpression, best detected by IHC, and this may not be associated with gene amplification (25). In our study' positive ***HER-2\neu*** Immunohistochemical staining was found in 41.6% (25 out of the total 60 cases) of the study group of urinary bladder carcinoma with a significant difference from that seen in the control group (P<0.05).This ensures that ***HER-2\neu*** has a fundamental role in the progression and proliferation of malignant cells. There was a positive correlation between the intensity of ***HER-2\neu*** immunohistochemical staining and histological

types of urinary bladder carcinoma but with significant difference among these 3 subtypes in relation to the HER-***2\neu*** over expression. This data was documented by other research (26) There was a gradual increase in the frequency of ***HER-2\neu*** overexpression in parallel with the increase in the grade of tumor (in grade I there was 35% ***HER-2\neu*** overexpression, in grade II 38.8% and in grade III 50%) with significant difference among the three degrees of differentiation ($p< 0.05$).This fact was proved by other studies(27,28). There is a significant difference between superficial low grade tumor (Ta , T1) and the invasive high grade tumor (T2 ,T3 and T4) ($P < 0.05$) but there is no significant differences between T2 ,T3 and T4 themselves .The immunohistochemical analysis Her-2\neu overexpression was reported in 42.85% of cases with recurrent cancer, while it was detected in 40% of those presented for the first time. The immunohistochemical analysis of the recurrent bladder cancer revealed that there is no significant difference between HER-2\neu overexpression with the pattern of tumor ($P > 0.05$). These finding are agrees with that reported by Tetu B, *et al* (29) who found that HER-2\neu overexpression was not associated with early tumor recurrence .In conclusion, Her-2/*neu* expression in urothelial cancers is variable and there is a correlation between Her-2/*neu* overexpression with a more aggressive clinical course& there is a significant relationship between total HER-2/neu overexpression and grade & stage of the bladder tumors. These findings further support the role of HER-2\neu in the carcinogenesis of bladder cancer regarding behavior, and aggressiveness of tumor especially transitional cell carcinoma and thus HER_2\neu could be considered as bad prognostic parameter in TCC. In which larger study focusing on immunohistochemical expression of HER-2\neu in squamous cell carcinoma of urinary bladder& further concurrent genetic DNA analysis of HER-2\neu gene in different histological types of bladder cancer patients are needed in the future.

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