An Immunohistochemical Study in Urothelial Carcinoma of HER2/neu in Iraqi Patients

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Abstract

Background: Bladder carcinoma is a common urological cancer. The most common type of bladder carcinoma in the United States is urothelial carcinoma (TCC).HER-2\neu proteins seems to include in pathogenesis of urothelial carcinoma. Its over expression has been associated with advanced stage of transitional cell carcinoma of bladder, this association has incited trials to HER2/neu targeted therapies in advanced diseases. Objectives: The aim of the current study was to evaluate the HER2 expression in UCB and its association with clinical and pathological factors. Materials and Methods: 50 cases with transitional cell carcinoma (13 female,37 male), were included in this study. Their ages ranged from 23 to 89 years, A group of 20 cases with benign bladder lesions (cystitis) was included as control group. Avidin-Biotin Complex (ABC) method was employed for immunohistochemical detection of HER-2\neu. Results: *HER-2\neu* over expression was found in 42% of transitional cell carcinoma, and no expression in benign bladder lesions (cystitis) was detected, (P value <0.05). HER-2\neu over expression was high in invasive lesions with stages (\geq T2) than in non-invasive stages (\leq T1) (*P* < 0.05). *HER-2\neu* over expression were well correlated to gender (P value<0.05) and age of patients (P value <0.05). Conclusions and Recommendations: From these findings we can conclude that HER-2\neu plays an important role in pathogenesis of transitional cell cancer evolution. This may support the evidence of its role in increased or uncontrolled proliferation of the malignant cells.

Keywords: HER2/neu, immunohistochemical expression, urothelial carcinoma

INTRODUCTION

Carcinoma of bladder is a destructive disease and a major reason of death worldwide.^[1] According to a cancer statistics, 2015, there are 74,000 new cases and more than 15,000 deaths of bladder cancer in the United States.^[2] While in Iraq, it is the fifth most common cancer; it is the second cancer in men and the tenth one in women according to Iraqi cancer registry 2011.^[3] Urothelial carcinoma represent the most common type and account for about 92% of all bladder cancerous tumor.^[4] At the time of diagnosis, approximately 20–40% of the cases were invasive tumor^[5]

Bladder carcinoma is common in men than women with a ratio of 3:1.^[6] Several parameters will use to determine the prognosis of bladder carcinoma such as age, grade, stage,^[7] lymph node status, HER 2/ neu and p53 expression.^[8] Actually, different consequences can be noticed in patients with the same grade and stage.^[9] Her2/neu is a transmembrane glycoprotein with tyrosine kinase activity encoded by Her2/neu proto-oncogene and belongs to epidermal growth factor receptor family.^[10]

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In normal situations, cells have low level of Her2 protein content in the membrane which is involved in the process of differentiation, proliferation and angiogenesis.^[11] Evaluation of Her2/neu overexpression is a very important prognostic factor for response to herceptin.^[12] in which the addition of antibody against Her2/neu protein (herceptin) to chemotherapy improved the overall survival rate of patients.^[13,14]

A broad mutability of HER2 expression in carcinoma of bladder which range from 6 to 80%, has been reported^[15] commonly associated with high grade and stage,^[16-18] while another studies have established no such association.^[15,19,20] Therefore, the value of HER2 overexpression in UCB is still unclear.

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The aim of the current study was to evaluate the HER2 expression in UCB and its association with clinical and pathological factors.

MATERIALS AND METHODS

This retrospective study was carried out in the college of Medicine – University of Babylon, Iraq from February 2018 to September 2020 and the specimens were collected from al Hilla teaching hospitals in Hila city.

In this study, fifty cases (37males and 13 females) with the transitional cell carcinoma were included, their ages were ranging from 23 to 89 years.

Twenty specimens of benign bladder lesions taken from patients with cystitis were considered as control group.

Sections of hematoxylin and eosin were examined to confirm the diagnosis and for tumor grading according to the WHO classification 2016^[21] and were evaluated for pathological stage according to TNM staging system tumors.^[22]

Positive control sections were processed parallel with each set of immunostaining.

Sections which were untreated with primary antibody (HER - $\ 2$ neu) had been considered as negative controls for each set of slides.

The Avidin Biotin Complex detection (ABC) system used on specimens of 5 micron, thick sections were cut from the formalin-fixed paraffin-embedded blocks and placed on positive charged slides.

Each set contains 5 slides of study group, 1 slide of control group (cystitis),1 positive control, and 1 slide of negative control.(all run in parallel at the same time).

Scoring system

Target score (Hercep Test Score)

The immunohistochemical staining was evaluated by *Truls G. et al.*, 2005.^[23] The Hercep Test Score using the 0, +1,+2,+3 scale.

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).

Qualitative assessment

Faint staining pattern, at the cell membrane, that could only be detected by using higher magnification (objective 40). While Strong staining pattern, easily seen by low magnification (objective 4).

Positive versus negative receptor expression

Score +2 and +3 were considered receptor positive and scores 0 and +1 receptor negative.

Statistical analyses

Statistical analyses of all results were carried out by the help of SPSS version 18 software statistical package using chi square (P value at level of significance less than 0.05).

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information were reviewed and approved by a local ethics committee according to the document number 645 on February 7, 2018.

RESULTS

Clinicopathological analysis

Fifty cases of transitional cell carcinoma and twenty cases of normal looking urothelial tissues were included in this study. The clinicopathological assessment revealed that 11(22%) of cases were ≤ 50 and 39(78%) were $\geq 50, 37(74\%)$ were males and 13 (26%) were females, 36(73.333%) were low grade and 14(26.666%) were high grade, 28(56%) were of \leq T1 and 22(44%) of \geq T2 [Table 1]

HER-2\neu immunohistochemical study

The results revealed that immunostaining of HER-2\neu protein was exclusively accumulated in the cell membrane of malignant cells.

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 21 (42%) out of 50 cases of transitional cell carcinoma [Figures 1 and 2] and 29 (58%) cases were negative with highly significant difference in comparison with control group (P < 0.05) [Table 2].

In our study, we found a significant association between HER-2 immunoexpression and tumour stage (*p* value = 0.025). HER-2/neu overexpression was found more frequently in muscle invasive tumor \geq T2 (57.14%), than in non-muscle invasive tumor \leq T1(42.85%) [Table 3]. Also there were a significant different between score of HER2/neu and tumor stage (p value=0.021) [Table 4].

Immunohistochemical analysis of HER-2\neu protein over expression in relation to grade of tumor revealed that positive HER-2\neu was reported in 14 (38.88%) cases

Table 1: The frequency and types of presented cases in this study					
Parameter	No. of cases	Percentage	Total		
Types of tissue					
Normal looking (control group) urothelial tissue	20	28.57%			
Urothelial carcinoma (study group)	50	71.42%	70		
Age					
≤50	11	22%	50		
>50	39	78%			
Gender			50		
Male	37	74%			
Female	13	26%	50		
Grade					
Low grade	36	73.333	50		
High grade	14	26.666			
Depth of invasion (T)					
≤T1	28	56%			
≥T2	22	44%			



Figure 1: Transitional cell carcinoma low grade showing positive HER-2\ neu receptor immunostaining score 2 (10X)

out of 36 low grade, and 7(50%) out of 14 of high grade, with a positive correlation between the detection rate of HER-2\neu immunostaining and grade of tumor, i.e., as tumor grade increase more HER-2\neu over expression but with no significant difference (P = 0.485) [Table 5]. The intensity of immunostaining of HER-2\neu protein was assessed in relation to the grade of tumor, and there is no significant difference between the intensity of HER-2\ neu and the grade of tumor (P > 0.05) [Table 6].

Regarding the gender there was a significant association between HER-2 over-expression and gender (p value = 0.002) [Table 7].

HER-2/neu over-expression was significantly correlated with patient's age (p value 0.004) [Table 8].

DISCUSSION

In the early 1990s, HER-2/neu protien received attention, in which several studies will aimed to define its role



Figure 2: Transitional cell carcinoma grade II showing positive HER-2\ neu receptor immunostaining score 3 (10X)

in urinary bladder TCC and its ability as a prognostic indicator.

Many authors were assessed HER2/neu overexpression in urothelial carcinomas, because the HER2/neu protein is implicated in pathogenesis of urothelial carcinoma, as important as in breast cancer^[24]

In our study we found over-expression of HER2/neu in 42% of cases. This result is lower than those of Nedjadi *et al.* 2016,El Gehani *et al.* 2012 and Naik DS *et al.* 2011,^[25-27] who found that the HER-2/neu over expression were 60%, 59% and 60%) of patients with bladder carcinoma respectively

Our results were higher than that reported by Al-Tereihi *et al*(2011).^[28] and Hammam *et al.* (2015),^[29] who found that over-expression of HER2/neu was 41.66% and 27% respectively.

These variability in the expression of HER2/neu may be due to variability in kits, solutions, differences in applied

Table 2: Immunohistochemical expression of HER -2 \setminus neu in benign and malignant bladder tissues				
Type of Tissue	limmunostaining of HER-2 \neu			
	Positive	Negative		
Benign (Cystitis)	0	20 100%	20	
Bladder carcinoma	21 42%	29 58%	50	
Total	21	49	70	

Table 3: HER-2\neu immunostaining in relation to stage (T) of tumor in transitional cell carcinoma patients

Immunostaining of HER2/neu	Stage		Total	P value
	≤ T1	≥ T2		
Positive	9 42.85%	12 57.14%	21	0.025
Negative	19 65.517%	10 34.482%	29	
Total	28 56%	22 44%	50	

Significant difference P value <0.05

Table 4: Correlation between intensity of HER-2\neu and stage (T) of transitional cell carcinoma

HER-2\neu intensity (Score)	Stage		Total	P value
	T1	T2		
0	15 65.217%	8 34.782%	23	0.021
+1	4 66.666%	2 33.333%	6	
+2	6 54.545%	5 45.454%	11	
+3	3 30%	7 70%	10	
Total	28	22	50	

Significant difference P value <0.05

Table 5: Immunostaining of HER 2\neu in relation to grade of tumor in transitional cell carcinoma				
Immunostaining of HER-2\neu	Grade		Total	P value
	Low grade	High grade		
Positive	14 66.666%	7 33.333%	21	0.485
Negative	22 75.862%	7 24.137%	29	
Total	36	14	50	
P > 0.05 not significant				

Table 6: Correlation between intensity of HER-2\neu and grade of transitional cell carcinoma HER-2\neu intensity (Score) Grade Total P value Low grade High grade 0 18 78.26% 5 21.73% 23 0.478 +14 66.66% 2 33.33% 6 +2 9 81%.81 2 18.18 11 +310 5 50% 5 50% 36 14 50 Total

P > 0.05 not significant

criteria for HER2 assessment and subject interpretation of staining result.

In our results, there is a significant difference between muscle invasive tumors and non-muscle invasive tumors in which the expression of HER2/neu is more in muscle invasive tumor(12 out of 21 of positive cases) (p value=0.025) [Table 3], also the intensity of HER2 increases as the

stage of the tumor increases, with significant difference between muscle invasive and non-muscle invasive tumors (P = 0.021) [Table 4]. These findings are in agreement with previous studies.^[25,30-33]

Regarding the grade of tumor, our results showed no variance in the expression of HER2/neu of high grading tumors in relative to low grading tumors (P = 0.485),

Table 7: Immunostaining of HER 2\neu in relation to gender					
Immunostaining of HER-2\neu	Gender		Total	P value	
	Female	Male			
Positive	2 9.523%	19 90.476%	21	0.002	
Negative	11 37.93%	18 62.06%	29		
Total	21	29	50		
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P < 0.05 significant(p=0.002)

Table 8: Immunostaining of HER 2\neu in relation to age						
Age	Immunostainin	Immunostaining of HER-2\neu		P value		
	Positive	Negative				
≤50	8 72.727%	3 27.272%	11	0.004		
>50	13 33.333%	26 66.666%	39			
Total	21	29	50			
P < 0.05 significan	t					

0.05 significant

in agreement with the report of Ioachim et al.[34] who found no relation between HER2 expression and grade of tumor, but in disagreement with other reports done by Jimenez et al, Kruger et al, [35,36] This difference results due to the heterogeneity between protocols, variant clones of primary antibodies, different in grading system and also different methods for scoring of HER2 /neu.

This study revealed that there is highly significant difference between male and female in relation to HER2/ neu expression in which 90.476% of positive cases were male and 9.523% of positive cases were female (p value=0.002), in disagreement with Ismail et al. and Samane et al^[4,37] who found no significant difference between gender in relation to HER2 expression,

Also there is highly significant difference (P = 0.004)between cases of ≤ 50 and cases of >50 in which 8 out of 11 (72.727%) of those ≤50 years were positive and only 13 out of 39 (33.333%) of those >50 years were positive., which is disagree with the study of El Gehani et al. and Mai M et al^[38,39] who fund no relationship between age and HER2 /neu expression

In conclusion HER2/neu overexpression was well correlated to the stage of tumor indicating that HER-2\ neu positive tumors are biologically aggressive and associated with poor prognosis.

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Conflicts of interest

There are no conflicts of interest.

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