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Immunohistochemical Study of HER-2/neu Expression in Urothelial Bladder Carcinoma

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Abstract: The current study aimed to assess HER-2/neu overexpression in sixty cases of urothelial bladder carcinomas by immunohistochemistry and in relation with their clinicopathologic features. Overexpression of HER-2/neu was seen in 29 (48.3%) cases [9 cases score +2 and 20 cases score +3], while 31(51.7%) cases were negative [16 cases score 0 and 15 cases score +1]. HER-2/neu overexpression in 27 (62.8%) high grade cases was statistically significant when compared with 2 (11.8%) low grade cases. The detection of HER-2/neu immunostaining was statistically insignificant in different pathological stage of studied cases, although HER-2/neu positivity tend to predominate in advanced pathological stage of tumor (T3 and T4). These findings may have clinical implications for the management of patients with HER-2/neu positive urothelial bladder carcinoma.

Key words: HER-2/neu · Immunohistochemistry · Urothelial Bladder Carcinoma

INTRODUCTION

Worldwide, bladder carcinoma is the ninth most common type of cancer with high incidence in industrialized countries [1]. It represent the fourth most common cancer in men and the eighth in women [2]. Besides cigarette smoking and certain occupational and industrial exposures, schistosomal infection is another major risk factor for bladder carcinoma, particularly in Egypt which has a high prevalence of this parasite [3].

According to WHO, Approximately 90 percent of epithelial bladder tumors are urothelial carcinomas [4], the formerly used term transitional cell carcinoma (TCC) is largely replaced by urothelial carcinoma, although urologists and pathologists still use both interchangeably [5].

The biological behavior of the bladder carcinomas is unpredictable and the morphological methods are often insufficient to predict the clinical outcome of disease. This fact has encouraged the researchs for prognostic markers [6]. The major tumor markers for prognosis of TCC are: oncogenes (p53, bcl-2, HER-2/neu) [7]; cyclin-dependent kinases and its inhibitors (cyclin D1, cyclin E, p21Wafl, p27Kipl);and others [8].

HER-2/neu, a 185 kDa transmembrane receptor tyrosine kinase, is a member of the epidermal growth factor receptor family localized to chromosome 17q [9]. It was first identified in 1981[10]. HER-2/neu play an important role in neoplastic cell growth in addition to its role in regulating normal cellular proliferation [3].

The assessment of HER2/neu overexpression is not a new idea, the first observations have been published by Zhau *et al*. [11] who reported HER-2/neu overexpression in bladder cancer of 70% their patients for the first time. Since then, several authors pointed that HER-2/neu expression in urothelial carcinomas had ranged from 2% to 74%[12].

Overexpression of HER-2/neu protein seemsto correlate with earlier tumor recurrence, worsened pathologic stage, tumor grade and decreased survival [13], although the prognostic role of HER2/neu has been controversial with several conflicting results in the literature [12] and may vary depending on associated chemotherapeutic regimens [13].

MATERIALS AND METHODS

Archival samples of 60 radical cystectomy urothelial bladder carcinomas were examined.Samples were collected

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from the Pathology Department of Kasr El Aini Hospital, Faculty of Medicine, Cairo University from 2009 to 2011, where clinicopathological features and representative paraffin blocks were obtained. The patients were 31 to 81 years old (mean, 61.1±9.8 years); 50 (83.3%) were male and 10(16.7%) were female. Histopathological grade and the TNM classification were evaluated based on WHO classification [4].

Immunohistochemical staining In brief, Paraffin sections, 4 µm thick were stained using a standardized Hercep test kit. The primary antibody is Monoclonal Mouse Anti-Human HER 2- Ab-17 from (Thermo Fisher Scientific company,UK). The staining procedure included an antigen retrieval step. Staining was performed according to the manufacturer's guidelines. Each immunohistochemical experiment included a set of positive and negative control slides.

Scoring of HER-2/neu overexpression, HER-2/neu positivity was semiquantitatively assessed and scored under light microscope. Only membrane staining (dark golden-brown precipitate) of malignant cells had evaluated. The cases with only cytoplasmic staining were considered negative, irrespective of the staining intensity. The intensity of HER-2/neu protein expression and percentage of staining cells were assessed and scored on a scale of 0 to 3+, using the following system: 1+(almost faint, equivocal and incomplete membranous staining); 2+ (unequivocal, complete membranous pattern, with moderate intensity); and 3+(complete and strong membranous pattern). Cases with a score of 2 or 3 were considered as HER-2/neu positive [6].

Statistical analysis For statistical analysis was carried out by Statistical Package for the Social Sciences (SPSS) version 16 for Windows software. The Chi-square ($x2$) test was used to detect statistically significant differences between the groups, with a significance level of ($p<0.05$).

RESULTS

In present study, HER-2/neu overexpression was reported in 29 (48.3%) out of 60 cases of urothelial carcinoma cases [9 cases score +2 and 20 cases score +3], while 31 (51.7%) cases were negative[16 cases score 0 and 15 cases score +1]. The present study revealed a significant correlation between HER-2/neu overexpression and the tumor grade ($p< 0.001$). Although there was statistically insignificant association between HER-2/neu positivity and tumor stage ($p=0.16$), the intensity of HER2-/neu positivity increased in advanced pathological (pT) stage of urothelial carcinoma in studied cases ($p=0.007$). HER-2/neu did not show any significant relation with sex,size, or lymph nodes status(pN) (Table1).

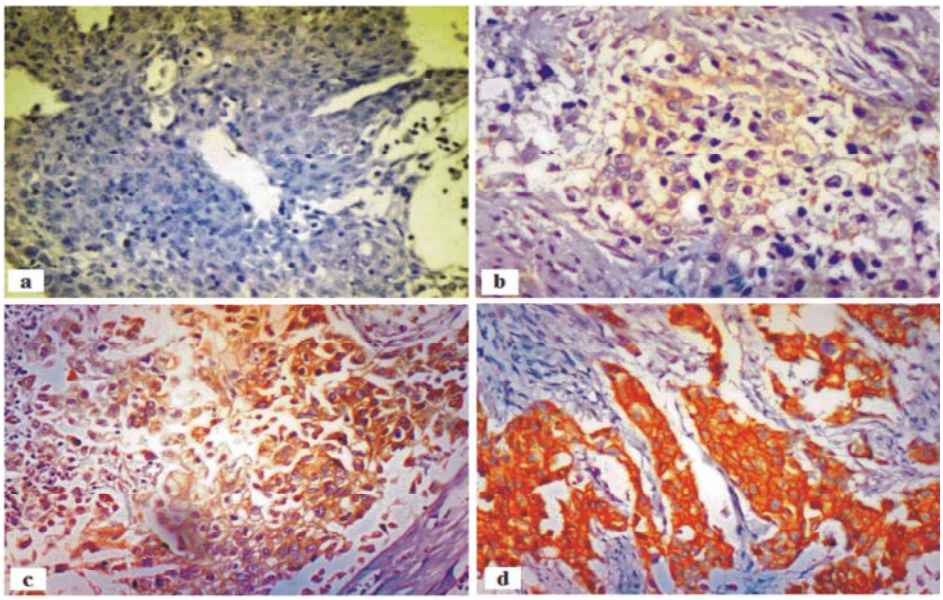

Fig. 1: Malignant cells of TCC tumor showed:-
(a) No HER2/neu immunostaining (Negative) X 200.
(b) Incomplete and faint membranous HER2/neu immunostaining score 1+ (Negative) X 200.
(c) Complete and moderate membranous immunostaining of HER2/neu score 2+ (positive) X 200.
(d) Intensely positive membranous immunostaining of HER2/neu (score 3+) X 200.

Table 1: Expression and intensity of HER2/neu expression in the studied urothelial carcinoma cases

	NO	Negative		Positive		
Parameter	%	Score 0	Score 1+	Score 2+	Score 3+	P value
Total	60	16	15	9	20	0.24
	100%	26.7%	25%	15%	33.3%	
Gender						
Male	50	13	13	8	16	0.9
	83.3%	26%	26%	16%	32%	
Female	10	3	2	1	4	
	16.7%	30%	20%	10%	40%	
Size of tumor						
≤3 cm	17	7	4	1	5	0.21
	28.3%	41.2%	23.5%	5.9%	29.4%	
>3 cm	43	9	11	8	15	
	71.7%	20.9%	25.6%	18.6%	34.9%	
Pathological grade						
Low grade	17	9	6	1	1	< 0.001*
	28.3%	52.9%	35.3%	5.9%	5.9%	
High grade	43	7	9	8	19	
	71.7%	16.3%	20.9%	18.6%	44.2%	
Pathological (pT)stage						
T1	2	1	1	0	0	0.007 *
	3.3 %	50%	50%	0%	0%	
T2a	2	2	0	0	0	
	3.3 %	100%	0%	0%	0%	
T2b	4	0	2	1	1	
	6.7 %	0%	50%	25%	25%	
T3a	4	1	1	2	0	
	6.7 %	25%	25%	50%	0%	
T3b	43	12	11	6	14	
	71.7%	27.9%	25.6%	14%	32.6%	
T4a	4	0	0	0	4	
	6.7 % %	0%	0%	0%	100%	
T4b	1	0	0	0	1	
	1.7%	0%	0%	0%	100%	
Pathological (pN) stage						
pNX	3	2	0	1	0	0.74
	5%	66.7%	0%	33.3%	0%	
pN0	31	6	9	4	12	
	51.7%	19.4%	29%	12.9%	38.7%	
pN1	12	4	3	2	3	
	20%	33.3%	25%	16.7%	25%	
pN2	14	4	3	2	5	
	23.3%	28.6%	21.4%	14.3%	35.7%	

DISCUSSION

The wide variations of HER-2/neu overexpression in bladder carcinoma of different studies are most likely explained by tumor heterogeneity, small number of collecting samples, specimen quality(fresh/formalin fixed), tumor grade and/or stage and cross-reactivity of antibodies with other epitopes. Furthermore the use of different detection methods (i.e. PCR, FSH and IHC), different type of kits and antibodies used in Immunohistochemistry as well as definition of HER-2/neu positivity [14-16]. Unlike breast cancer, overexpression without gene amplification is more common in urothelial bladder carcinoma [17].

In the current study, a HER-2/neu positivity was detected in 48.3% of urothelial carcinoma cases, this rate is within the range of previously published data [12]. Our results is coordinated with that obtained in previous studies [3, 15, 18]. While a higher rates of HER-2/neu overexpression were obtained by Hammam *et al*. [19] Gandour-Edwards *et al*. [20] who reported that HER-2/neu positivity in 72.2% and 71% of their primary TCC cases respectively. Others [16, 21-24] have recorded lower results of HER-2/neu expression. Also less figure than ours was fulfilled by Lae *et al*. [17] who studied 1005 TCC patients, they recorded HER-2/neu overexpression in 93 (9.2%) tumors. Moreover, two studies previously conducted by Krüger *et al*. [25] and Naruse *et al*. [26],

the authors considered only score 3+ cases were positive and registered 21.7% and 41.3% for HER-2/neu protein expression by Immunohistochemistry respectively.

We clarified that HER-2/neu overexpression in (62.8%) of high grade tumors was statistically significant when compared with (11.8%) of low grade (P<0.001). However our series indicated that, there was no significant correlation between HER-2/neu expression and different pathological (pT) stage of studied cases, we found significant relationship between the intensity of HER-2/neu positivity and pT stage of tumor (P=0.007) as most of HER-2/neu overexpression cases were in advanced pT stages. In compatible with these outcomes, previous studies [15, 18, 25] detected that there was a significant direct relationship between HER-2/neu overexpression and grade of the bladder TCC tumors and a tendency for HER-2/neu positive status was more likely to predominate in advanced stage (pT) tumors.

In contrast to our current study, the prior two studies done by Matsubara [23] and Naruse *et al*. [26]. The authors concluded that HER-2/neu positivity to show significant direct relationship with tumor stage. Unlike to our findings, several studies [16, 19, 22] categorized their cases as superficial and invasive tumors demonstrated that HER2/neu overexpression was statistically significant with both tumor stage and grade. In other studies [12, 27-29] there was no significant association between HER-2 /neu expression and either pathological staging or tumor grading of urothelial bladder carcinoma.

We found there were no statistically significant differences between different lymph node status (pN) and HER-2/neu immunostaining.This is in agreement with previous studies [12, 25, 26], where the authors indicated that there was no correlation between HER-2/neu positive status and the presence of lymph node metastasis. In contrast kolla *et al*. [15] recorded a significant relationship.

In the present work, no significant correlation was seen between the HER-2/neu overexpression and the size of tumor. This in agreement with Chow *et al*. [18] who reported that HER-2/neu protein overexpression is not related to tumor size of their studied TCC cases.

CONCLUSION

The present study demonstrates that expression of HER-2/neu has a direct relationship with the grade and mostly predominate in advanced pT stage. Assessment of HER-2/neu status can be used to identify patients who may benefit from adjuvant HER-2/neu targeted therapy after radical cystectomy.

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