

# Overexpression of *HER-2/neu* Oncogene and Transitional Cell Carcinoma of Bladder

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**Introduction:** The aim of this study was to evaluate the relationship between *HER-2/neu* oncogene expression and grade of transitional cell carcinoma (TCC) of the bladder.

**Materials and Methods:** In this cross-sectional study, 75 formalin-fixed paraffin-embedded specimens of primary TCC of the bladder were stained with a monoclonal antibody against *HER-2/neu* oncoprotein. Another section was stained by hematoxylin-eosin and the tumor grade was determined according to the World Health Organization/International Society of Urological Pathologists criteria.

**Results:** Tumor specimens belonged to 49 men (65.3%) and 26 women (34.7%) with a mean age of  $56.3 \pm 9.1$  years (range, 39 to 80 years). The tumor grades were 1, 2, and 3 in 14 (18.7%), 35 (46.7%), and 26 (34.7%) specimens, respectively. A total of 28 (37.3%) patients were positive for overexpression of *HER-2/neu*. There were 1 case of *HER-2/neu*-positive (7.1%) with a grade 1 tumor, 10 (28.6%) with grade 2, and 17 (65.4%) with grade 3; a significant relationship between *HER-2/neu* overexpression and grade of the bladder TCC tumors was found ( $P = .002$ ).

**Conclusion:** Expression of *HER-2/neu* oncogene has a direct relationship with the grade of the bladder TCC. Further studies with longer follow-up period and a larger sample size can determine the probable role of *HER-2/neu* expression as a prognostic factor in the TCC of bladder.

Keywords: bladder, transitional cell carcinoma, grade, *HER-2/neu*, oncogene

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## INTRODUCTION

It is estimated that 67 160 new cases of transitional cell carcinoma (TCC) of the bladder and 13 750 deaths will occur in 2007 in the United States due to this disease. For local tumors and distant metastases, the 5-year survival rates are 48% and 6%, respectively.<sup>(1)</sup>

Life expectancy will increase in patients with TCC if a correct and early diagnosis is made. Several factors involve in determination of prognosis and selection of the treatment. A known independent factor is the grade of the tumor.<sup>(1,2)</sup> Other factors such as growth rate, patient's age, and tumor aggressiveness are also

important.<sup>(3)</sup> However, they may be inadequate in determination of the prognosis and therefore, other factors such as oncoproteins can be helpful.<sup>(4)</sup> Molecular predictors of cancer behavior are not currently available for bladder TCC and would be valuable to categorize patients accurately. *HER-2/neu* (*c-erb B2*) is an oncogene encoding a type 1 tyrosine kinase growth factor receptor. Latif and colleagues reported that polysomy 17, gene amplification, and *HER-2/neu* overexpression are associated with a poor prognosis in patients with bladder TCC.<sup>(4)</sup> Overexpression of *HER-2/neu* has been associated with some different types of human

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cancers.<sup>(5,6)</sup> In the present study, we evaluated the expression of this gene in bladder TCC and its relationship with tumor grade.

## MATERIALS AND METHODS

We evaluated the available tissue blocks of 75 patients with bladder TCC who had referred to Mostafa Khomeini Hospital between 2001 and 2004. Three-micrometer thick sections were prepared from paraffin-embedded tissue blocks and stained by hematoxylin-eosin method. Tumor grade was then determined using the World Health Organization/International Society of Urological Pathologists criteria by a single pathologist blinded to the records of the patients.<sup>(7)</sup>

A manual avidine-biotin-peroxidase complex procedure was used in the immunohistochemical analysis (DakoCytomation, Copenhagen, Denmark); another section from each block was used for evaluation of *HER-2/neu* oncoprotein. For this purpose, the sections were deparaffinized and processed as follows: the specimens were first placed in oven at 50°C to 60°C for 30 minutes, and then, were rinsed in 100% xylol, 100%, 85%, and 75% ethanol, water, 10% phosphate-buffered saline (PBS), 1:9 H<sub>2</sub>O<sub>2</sub>/ethanol solution (for 10 minutes), and 10% PBS. The specimens were then placed in sodium nitrate buffer (pH = 8.0) and autoclave for 10 minutes in 120°C and pressure of 1.2 atmosphere. Afterwards, they were rinsed in 10% PBS again. Two drops of serum blocking solution was then added, and after 10 minutes, the slides were covered by 2 drops of *HER-2/neu* primary antibody for 30 to 60 minutes and rinsed in 10% PBS. Two drops of biotinylated antibody were added on the slides and after 10 minutes, rinsed in 10% PBS. Then, 2 drops of enzyme conjugate was added and after 10 minutes, rinsed in 10% PBS. Thereafter, 100 Lambda ready DAB chromogen was added and remained for 50 minutes and then the slides were rinsed in 10% PBS. Two drops of hematoxylin was added for contrast making for 1 to 3 minutes and rinsed in water and PBS for 30 seconds, dehydrated in 75%, 85%, and 100% alcohol, and then 100% xylol for clearing. The cover was slipped and coded. The slides were then evaluated under standard light microscope with × 40 magnification and the positive cases for overexpression of *HER-2/neu* were determined.

The membrane staining intensity and pattern were considered for scoring according to the Food and Drug Administration approved criteria<sup>(8)</sup>: zero, no staining or membrane staining observed in less than 10% of the tumor cells; 1+, partial membrane staining in more than 10% of the tumor cells and membrane staining not circumferential; 2+, circumferential weak to moderate staining observed in more than 10% of the tumor cells; 3+, strong circumferential membrane staining observed in more than 10% of the tumor cells. Areas that were poorly preserved, crushed, cauterized, folded, or retracted were specifically avoided. Scores of 2+ and 3+ were considered positive.

Data were analyzed using the SPSS software (Statistical Package for the Social Sciences, version 13.0, SPSS Inc, Chicago, Ill, USA) and the chi-square was used to compare dichotomous variables between tumor grade groups.

## RESULTS

Of the patients, 49 (65.3%) were men and 26 (34.7%) were women. The mean age of the patients was 56.3 ± 9.1 years (range, 39 to 80 years). The tumors were grade 1, 2, and 3 in 14 (18.7%), 35 (46.7%), and 26 (34.7%) cases, respectively. A total of 28 (37.3%) patients were positive for overexpression of *HER-2/neu* oncogene.

High histologic grades of the TCC tumors were associated with increased expression of *HER-2/neu*. There were 1 case of *HER-2/neu*-positive (7.1%) with a grade 1 tumor, 10 (28.6%) with grade 2, and 17 (65.4%) with grade 3 ( $P = .002$ ).

## DISCUSSION

According to our findings, higher grade of the TCC of the bladder is accompanied by *HER-2/neu* overexpression. In the study by Latif and colleagues, *HER-2/neu* and muscle invasiveness were evaluated in 25 patients with TCC. No significant relation was detected in the expression of this protooncogene and grade of the tumors; however, the percentage of positive cells for *HER-2/neu* was greater in invasive tumors suggesting anti-*HER-2/neu* treatment in invasive cases.<sup>(4)</sup> Our sample size was greater, but their method was more sensitive since they used fluorescence in situ hybridization for

immunohistochemistry. In another study performed on a total of 106 patients, it was revealed that higher grade and stage of the TCC tumor correlated with more *HER-2/neu* overexpression.<sup>(6)</sup> In the latter study, stage of the tumors and *HER-2/neu* expression were both finally considered as 2 independent factors for disease-free survival. They also studied *p53* and *MDM2* overexpressions, but the correlation between these 2 markers and TCC grade and stage were not significant. These results agree with the results of Mellon and colleagues and Coombs and colleagues<sup>(9,10)</sup>; however, their results cannot be compared to ours due to different degrees of involvement.

One relevant study by Lipponen and associates on 91 patients with bladder TCC showed 11 patients (12%) to be *HER-2/neu*-positive and in 4% of them, the expression was graded as moderate or severe.<sup>(11)</sup> The expression of *HER-2/neu* was significantly related to the tumor grade according to the World Health Organization/International Society of Urological Pathologists criteria, whereas no significant difference was detected in its expression between the superficial and invasive or papillary and nonpapillary TCC tumors. In conclusion, they found out that moderate and severe overexpression of *HER-2/neu* oncoprotein in TCC seemed to be related to a more aggressive behavior of the tumor, while low expression of this oncoprotein had no predictive value.<sup>(11)</sup> Although that study had some differences in the method of cellular evaluation, it was similar in other ways to ours.

Tetu and coworkers studied low malignant potential papillary TCC tumors and found out that *HER-2/neu* expression was unremarkable in superficial bladder cancer which is compatible with our results.<sup>(12)</sup>

Coogan and colleagues' study on 54 selected paraffin blocks revealed *HER-2/neu* overexpression in 26% of the TCC cases; moreover, there were similarities between their results and ours in term of differences in overexpression and grade of malignancy. The difference in *HER-2/neu* overexpression rate between these 2 studies can be due to the small volume and selected nature of Coogan and colleagues' study sample.<sup>(13)</sup> In another study, 36% of cases were positive for *HER-2/neu* using the immunohistochemistry method precisely similar to our study and might confirm our results.<sup>(14)</sup>

## CONCLUSION

Since most previous studies have shown a relationship between the tumor grade and expression of *HER-2/neu* oncoprotein, as we did in the present study, this gene can be used in determination of the prognosis of bladder TCC. Finally, comprehensive research with longer follow-up period and larger sample sizes are needed for further elucidation of the role of the oncogenes.

## CONFLICT OF INTEREST

None declared.

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