

# Immunohistochemical study of Her2/neu marker status in urothelial tumors of bladder

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## Abstract

**Background:** Her2/neu is a marker of established clinical significance in the context of Her2/neu targeted therapy for breast carcinomas and gastric carcinomas. The present study was done to analyze the expression of Her2/neu in urothelial tumors of bladder in correlation with parameters like invasion, histological type and grade. **Materials and methods:** The retrospective study included 50 histologically diagnosed cases of urothelial tumors. The clinical and pathological findings were recorded. Immunohistochemical study was performed with Her2/neu marker on all cases and the findings were independently scored by two observers and consensus reached in case of any discrepancy. **Results:** The 50 cases of urothelial tumors included an age range of 40-90 years with a male: female ratio of 4:1. There were 40 cases (80%) of papillary tumors and 10 cases (20%) of non-papillary urothelial tumors. There was equal incidence of invasive urothelial carcinomas (50%) and non-invasive papillary urothelial carcinomas (50%). The Her2/neu positive expression (3+) was observed in 16 cases (32%) of the tumors. Equivocal results (2+) was identified in 11 cases (22%) and 23 cases (46%) were negative respectively. **Conclusion:** The incidence of Her2/neu positive expression (32%) in this study is comparable with other studies. The association with grade of tumors was found to be of some significance.


**Key Words:** Urothelial tumors, Bladder, Her2/neu.

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Received Date: 13/11/2016 Revised Date: 15/12/2016 Accepted Date: 23/01/2017

Access this article online	
Quick Response Code:	Website: <a href="http://www.medpulse.in">www.medpulse.in</a>
	DOI: 18 February 2017

## INTRODUCTION

Bladder cancer is the 7<sup>th</sup> most common cancer accounting for 3.2% of all cancers worldwide. Urothelial carcinoma constitutes more than 90% of bladder cancer cases.<sup>1</sup> Among the primary urothelial bladder tumors, 70 to 80% of patients present with either papillary noninvasive disease (pTa) or invasion limited to lamina propria (pT1), whereas only 20% present with muscle invasive forms ( $\geq$ pT2).<sup>2</sup> It is observed that 50-70% of new cancer cases

recur within 5 years with great likelihood of progression into aggressive, muscle invasive and metastatic forms.<sup>3</sup> Thus, despite advancement in treatment modalities, management of these tumours pose challenge for clinicians.

Her2/neu biomarker testing has been of established clinical significance in breast carcinomas<sup>4</sup> and gastric carcinomas.<sup>5</sup> ERBB family of receptor tyrosine kinases is found to be also altered at significant frequency in bladder cancer. EGFR amplification was observed in 11%, ERBB2 was found mutated in 5% and amplified in 7%, and ERBB3 was mutated in 11% and amplified in 2% of bladder cancers.<sup>6</sup> The c-erbB2 gene encodes the second variant of the epidermal growth factor receptor (EGFR), Her2/neu. This is a defective transmembrane tyrosine kinase receptor which is found to be involved in the control of epithelial cell growth and differentiation. Indian studies related to Her2/neu expression status in bladder tumors are few.<sup>7</sup> The present study was done to explore Her2/neu expression status in urothelial tumors of bladder in correlation with pathological parameters.

## MATERIALS AND METHODS

A retrospective study was done on 50 histopathologically diagnosed cases of urothelial tumors excluding the benign tumors. The study included mainly TURBT/Biopsy (44) and cystectomy (6) specimens. Clinical data was recorded from the files. The HandE slides were retrieved from the archives of the Department of Pathology and histomorphological features were analyzed. Tumors were classified and graded according to the 2016 WHO Classification of Tumours of the Urinary System.<sup>8</sup> One formalin-fixed paraffin-embedded tissue block and standard 4µm sections were subjected to immunohistochemical (IHC) study. Primary antibody to Her2/neu (code-EP3, Rabbit Monoclonal Antibody, Pathnsitu) was used. The Polyexcel HRP (non-biotin, micro-polymer based) DAB detection system was used with adequate positive and negative controls. The IHC study findings were scored by two independent observers with consensus reached in any discrepancy.

*Scoring and Interpretation of IHC results*<sup>9</sup>: Her2/neu staining was scored as; score 0 (negative) = no staining is observed or membrane staining that is incomplete and is faint/barely perceptible and within ≤10% of the tumor cells; score 1+ (negative) = incomplete membrane staining that is faint/barely perceptible and within >10% of the tumor cells; score 2+ (equivocal) = circumferential membrane staining that is incomplete and/or weak/moderate and within 10% of tumor cells or complete and circumferential membrane staining that is intense and within ≤ 10% of tumor cells; score 3+ (positive) = circumferential membrane staining that is complete intense and within >10% of tumor cells.

*Statistical analysis*: Frequency tables were analyzed using the Chi-square test to assess the significance of the correlation between the categorical variables, using a significance level of  $p < 0.05$ .

## RESULTS

The 50 cases of urothelial tumors of bladder studied were of age range (40-90) years, with a mean age of occurrence being 61.4 years. The male: female ratio was 4:1 with male preponderance. There was equal incidence of invasive carcinomas (50%) and non-invasive carcinomas (50%). The histological types of invasive carcinomas included mainly transitional cell carcinoma/urothelial carcinoma (21cases) with one case of sarcomatoid variant and three cases of divergent (glandular) differentiation. There were 40 cases (80%) of papillary carcinomas and 10 cases (20%) of non-papillary carcinomas. Papillary carcinomas included non-invasive (25) and invasive (15) tumors. In the non-invasive carcinomas there were both papillary carcinomas of low grade (17 cases) and high grade (8 cases). All the invasive carcinomas were high grade tumors.

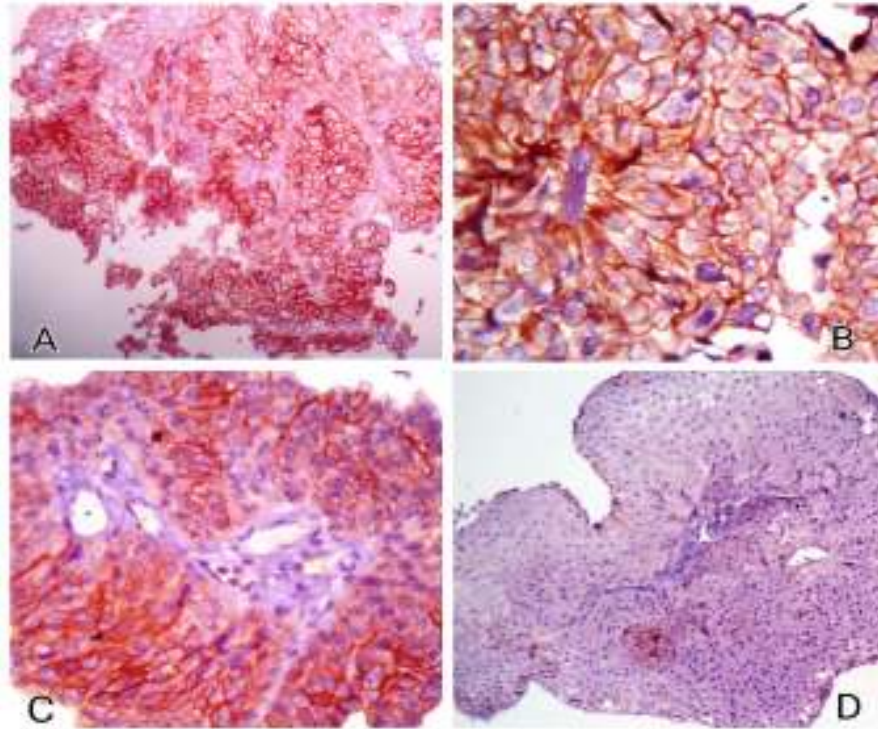
In the total of 50 cases studied, the positive Her2/neu expression (score 3+) was observed in 16 cases (32%) of the tumor. Equivocal results (score 2+) was identified in 11 cases (22%) and 23 cases (46%) were negative respectively. The papillary tumors were commonly observed in the study, with 14 cases (28%) of papillary tumors showing Her2/neu positivity compared to non-papillary tumors (4%). Among the invasive tumors 9 (18%) cases showed Her2/neu positivity and 7 (14%) cases of non-invasive were also positive. On comparison of grade and Her2/neu expression status, 10 cases (20%) of high grade tumors were positive with 6 (12%) of low grade tumors being positive.

The statistical analysis done to observe the significance of association between Her2/neu status and other pathological factors like invasion, grade and papillary morphology revealed statistically significant correlation with grade. (Table 1).

**Table 1:** Cross tabulation of her2/neu status with histological type, grade and invasion among total study subjects

	Her2/neu status			Total
	Positive (%)	Equivocal (%)	Negative (%)	
Papillary tumors	14 (28%)	10 (20%)	16 (32%)	<b>40 (80%)</b>
Non Papillary tumors	2 (4%)	1 (2%)	7 (14%)	<b>10 (20%)</b>
<b>Total</b>	<b>16 (32%)</b>	<b>11 (22%)</b>	<b>23 (46%)</b>	<b>50 (100%)</b>
<b>p - 0.229 (Not Significant)</b>				
High Grade	10 (20%)	4 (8%)	19 (38%)	<b>33 (66%)</b>
Low Grade	6 (12%)	7 (14%)	4 (8%)	<b>17 (34%)</b>
<b>Total</b>	<b>16 (32%)</b>	<b>11 (22%)</b>	<b>23 (46%)</b>	<b>50 (100%)</b>
<b>p- 0.026 (Significant)</b>				
Invasive	9 (18%)	2 (4%)	14 (28%)	<b>25 (50%)</b>
Non Invasive	7 (14 %)	9 (18%)	9 (18%)	<b>25 (50%)</b>
<b>Total</b>	<b>16 (32%)</b>	<b>11 (22%)</b>	<b>23 (46%)</b>	<b>50 (100%)</b>

p=0.055(Not Significant)



**Figure 1:** (A) Photomicrograph of Her2/neu positive immunostaining in infiltrating urothelial carcinoma showing intense membrane positivity in more than 10% of cells X10. (B) Photomicrograph of Her2/neu positive immunostaining in non-invasive high grade papillary neoplasm X40. (C) Photomicrograph of Her2/neu positive immunostaining in low grade papillary carcinoma X40. (D) Photomicrograph of noninvasive low grade papillary neoplasm with negative Her2/neu immunostaining X10

## DISCUSSION

The understanding of molecular biology of bladder cancer reveals specific genetic alterations and molecular types of tumors. This information is explored for studies on identification of biomarkers for diagnosis, disease monitoring, prediction of prognosis, response to therapy and targets for drug design.<sup>6,10</sup> Her2/neu, is one such marker of prognostic and predictive value that has been studied in many tumors to explore the clinical utility. Amplification and overexpression of Her2/neu in bladder carcinoma was first studied by Zhau *et al* in 1990. Studies on transitional bladder carcinoma demonstrated Her2 protein overexpression in the wide range of 17% to 76%.<sup>11</sup> Her2/neu gene is found to be amplified in 10-20% and overexpressed in 10-50% of invasively growing bladder cancer.<sup>1</sup> In this study 32% of urothelial carcinoma cases showed Her2/neu positivity which was comparable with some of the studies.<sup>12-14</sup> The difference in the incidence of Her2/neu positive expression among various studies may be attributed to differences in the study sample, methods and interpretation of IHC results. Some of the studies considered both score 2+ and 3+ to be positive<sup>7,14</sup> whereas others considered score 3+ as positive similar to our study.<sup>2,15</sup>

The present study had 22% of cases with equivocal results, the Her-2/neu gene amplification studies in these cases could be potential positive. Heterogeneous pattern of IHC staining was observed in some of the studies with negative and Her2/neu strongly positive areas. Heterogeneity was defined as at least one Her2 negative field in Her2 positive tumor.<sup>11, 14</sup> Some studies have also found variable score areas in a Her2/neu positive tumor. In the present study we had occasional case with such heterogeneous pattern. Bladder carcinomas are categorized mainly as non-invasive, superficial/non-muscle invasive, muscle invasive tumors and metastatic disease. In the present study there was equal incidence of non-invasive and invasive tumors. Incidence of Her2/neu overexpression in muscle-invasive urothelial bladder carcinoma is variable in the literature. It occurs in range from 4% to 32% in invasive stages.<sup>11</sup> In the present study near equal incidence of Her2/neu positivity was seen in both invasive (18%) and noninvasive (14%) cases with no significant difference. In the invasive tumor group 36% (9/25) of the tumors were Her2 positive. Transitional cell carcinoma (TCC)/Urothelial carcinoma is the usual histological type of invasive bladder tumor as also observed in present study. Rest of the tumors mostly exhibit variant morphology or mixed features (divergent differentiation). The other histological types like

squamous cell carcinoma and adenocarcinomas are rare.<sup>8</sup> In the present study most (80%) of the tumors were papillary urothelial carcinomas and incidence of Her2/neu positive tumors were also more (28%) in papillary compared to non-papillary (4%). The present study included only urothelial carcinomas and its variants. In one study of Her2/neu expression in variants of urothelial carcinomas, positive expression(3+) was seen in 56% of micropapillary urothelial carcinomas whereas the glandular, small cell or sarcomatoid variants did not show overexpression.<sup>15</sup> In the present study also the sarcomatoid variant and glandular differentiation cases did not show Her2 positive expression.

Multifocality and frequent recurrence is characteristic of urothelial tumors. Pathologic parameters such as tumour grade, stage, lympho vascular invasion are the factors that provide prognostic information. The prediction of risk of recurrence and or progression is of importance especially for superficial tumors. The role of Her2/neu as prognostic marker observed in most studies reveals the ambiguity. In some studies Her2/neu overexpression has been found to be correlating with increased risk of recurrence, lympho vascular invasion and poor prognosis.<sup>2,12</sup>

In other studies the Her2/neu over expression correlated with high grade and stage of tumors.<sup>2,13,16</sup> In contrary some studies had observed no significant correlation with these parameters.<sup>7,14</sup> In this study, the correlation of Her2/neu expression status and tumor grade was found to be significant (p -0.026). In another study of comparative analysis of primary and lymph node (LN) metastatic tumors, nearly all of the primary Her2 positive metastatic tumors of lymph node were Her2/neu positive. However, interestingly 45% of primary Her2/neu negative tumors, had Her2/neu positive lymph nodes.<sup>14</sup>

Success of Her2/neu targeted therapy for breast carcinoma has evoked interest in exploring similar role of Her2/neu testing in patients with bladder cancer. Presently, advances in the management of muscle invasive bladder cancer are aimed at bladder preserving strategies. Thus, multiple targets are being evaluated including Her2/neu, in various stages of bladder cancer by studies either as a monotherapy or in combination with cytotoxic chemotherapeutic agents.<sup>10</sup>

## CONCLUSION

The Her2/neu positive expression rate (32%) in this study is comparable with other studies. We comparatively observed frequent Her2/neu positive expression in invasive urothelial carcinomas of high grade. However, there was no significant difference in the noninvasive and invasive groups. The association with grade of tumor was found to be of significance.

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Source of Support: None Declared  
Conflict of Interest: None Declared