

**A Clinicopathological Study of Urothelial Carcinoma and Its Correlation with Her2/Neu Expression.**

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**Type of Publication:** Original Research Article

**Conflicts of Interest:** Nil

**Abstract**

**Introduction:** Urothelial Carcinoma is one of the most prevalent cancers in men. Patients presented with varied symptoms like hematuria, dysuria, urgency, frequency. HER2/neu expressions have found to be related to many cancers like breast, prostate, stomach. Despite many reports in the literature the prognostic significance of its overexpression in urothelial carcinoma remains unclear.

**Aim:** To study the relationship between tumour grade and Clinical Presentation and correlation of HER2/neu with tumour grade.

**Material and Methods:** 117 cases of urothelial carcinoma were diagnosed in our department between January 2016 and July 2017. The tumour specimens obtained by transurethral resection and cystectomy were evaluated by immunohistochemistry using HER2/neu antibody.

**Result** HER2/neu overexpression was present in 78/117 (66.66%) of cases with 60/75 (80%) in high grade tumours and 18/42 (42.85%) in low grade tumours.

**Conclusion:** There was a significant correlation between HER2/neu expression and grade of the tumour. Thus this knowledge can be used for Targeted therapy in future, but no correlation was found between clinical features and Grade of the tumour.

**Keywords:** Urothelial Carcinoma, HER2/neu expression

**Introduction**

Bladder cancer is the fourth most common malignancy, in men and the tenth most common in women. It is the eighth leading cause of death in men.<sup>1</sup>

The bladder is a common site of cancer development in urinary tract.

At the time of diagnosis, around 80% of the urothelial bladder carcinomas are superficial and 20% of them are invasive. Tumours that invade the deep muscle layer of bladder are assigned stage T2, while T3 and T4 lesions invade the perivesicle tissue and local structures respectively.

Approximately 10-15% of superficial or locally invasive (pTa/pT1) tumours progress to muscle invasion, and this risk is dependent on tumour stage and grade.

The Her2/neu oncogene is located on chromosome 17q11-21 and encodes for a tyrosine kinase trans-membrane growth factor receptor. Activation of the her2/neu receptor, following autophosphorylation of the tyrosine kinase residues results in the activation of cascade of intracellular proteins. Ultimately, the mitotic activity and metastatic potential of the cell increases.

Her2/neu expression in the bladder carcinoma is variable between different studies ranging between 9 to 81%.

It has been previously reported that 28% of the primary bladder cancers over- express her2/neu by immunohistochemistry (IHC) and that the primary tumour over- expression consistently predicts over-expression in a distant or regional metastatic site. However, 45% of Her2-negative primary tumours may show over- expression in their corresponding metastasis. These data suggested that Her2/neu might play a role in the biological progression of bladder cancer and the development of metastatic disease.

The HER 2-neu is known to contribute to physiologic mechanisms of cell proliferation by intrinsic tyrosine kinase activity.

So this current study is intended to find out over expression of HER 2-neu in Urothelial carcinoma of bladder and its association with clinical and pathological factors with the intension that this knowledge may be used for targeted therapy in future.

## Materials and Method

### Patients and Tissue Specimen

The present study was a observational and cross sectional study carried out in compliance with protocol in the department of pathology, R. G. Kar Medical College and Hospital, Kolkata (Tertiary care hospital in eastern India), after approval from the institutional ethics committee the study was conducted for a period of 18 months from January 2016 to July 2017. The tumour specimens were obtained from 92 transurethral resections and 25 cystectomies. All bladder tumour samples of patients who have received Chemotherapy or BCG , Recurrence of previously treated bladder tumours, Bladder tumours other than transional cell carcinoma, invasion and metastasis of other tumours to bladder were excluded from the study.

Four micrometer thick sections were stained with hematoxylin- eosin and reviewed. Staging was performed using the seventh edition of tumour-node-metastasis

classification. The 2016 world health organization classification was used for tumour grading. Clinicopathological Data including age, sex, clinical symptoms was collected.

### Immunohistochemistry

The hercepTest kit (Cell Marque HER/2neu CB-11) was used for HER2 protein expression analysis according to the manufacturer's instructions. Briefly, deparaffinized tissue sections were first incubated in a microwave for 5 min on high power. Peroxidase-blocking solution was used for 5min to prevent nonspecific immunostaining. Subsequently, the sections were incubated with primary antibody, anti mouse monoclonal HER2 for 60 min at 37<sup>0</sup> and followed by Secondary antibody for 30 min. The reaction product was visualized by the chromogen DAB, then counter staining with hematoxylin performed. A negative control without the primary antibody and a positive control of known breast carcinoma antigen was put up side by side.

Interpretation of immunostating was done by subjectively counting 1000 cells selecting the maximum positive area with strongest positive intensity and expressing it in percentage. For scoring , we used the recommendations of the American Society of clinical oncology/college of American Pathologists.

### Statistics

Statitl analysis was performed with the SPSS software( Statistical Package for Social Sciences, version 25) Spearman's analysis was done to evaluate the statistical significance of the association between tumour grade and HER2/neu expression and tumour grade and clinical features. Statistical significance was considered if P value was < 0.05.

### Results

In the present study, 117 cases of Urothelial Carcinoma were included

The median age of the patients was 65 years. The patients included 92 men (78.6%) and the sex ratio was 3:1. High Grade was noted in 75 cases (64.1%). Increased HER2/neu expression was present in 78 (66.66 %) of 117 cases, which included both High grade and Low grade tumours.

Amongst the High grade tumours, Her 2-neu over expression was seen in 60/75 (80%) cases. Score 0 was seen in 6/75 cases. Score +1 was seen in 9/75 cases, Score +2 was seen in 15/725 cases, Score +3 was seen in 45/75 cases.

In Low grade tumours Her 2-neu over expression was seen in 18/42 (42.85%) cases .Score 0 was seen in 7/42 cases, Score +1 was seen in 17/42 cases, Score +2 was seen in 9/42 cases and Score +3 was seen in 9/42 cases. Overall Her2-neu over expression was seen in 78/117 (66.66%) cases.

In this study more than 90% patients presented with hematuria as the most common symptom. Rest of the symptoms included Frequency, urgency, dysuria and one case presented with urinary retention.

Table 1: Clinicopathological Characteristics

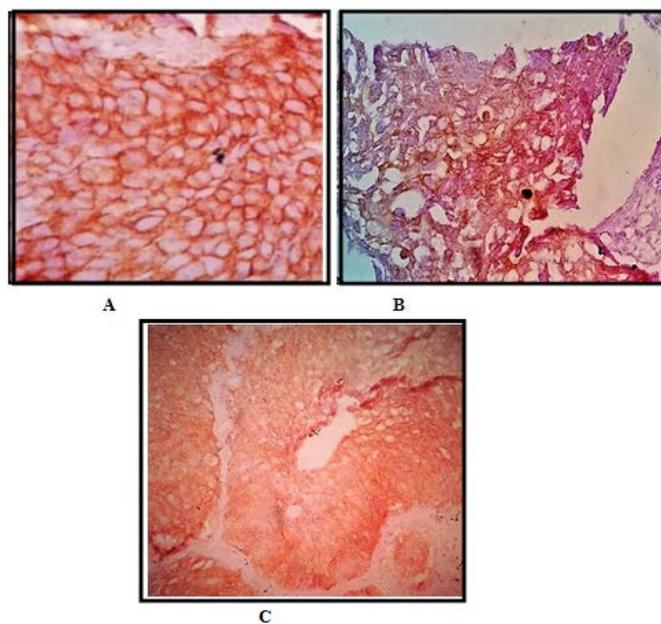
Characteristics	NO (%) of cases
<b>Sex</b>	
Male	92 (78.6)
Female	25 (21.3)
<b>Tumour Grade</b>	
Low	42 (35.8)
High	75 (64.1)
<b>HER2 protein expression</b>	
0	11 (9.4)
1+	24 (20.5)
2+	25 (21.3)
3+	54 (46.1)
<b>Clinical Features</b>	
Hematuria	105
Dysuria	56
Urgency	70
Frequency	30
Urinary Retention	1

Spearman’s analysis was done to compare clinical features with microscopic features. Comparison between these two features was not statistically significant with a  $p > 0.05$ . From this comparison we came to a conclusion that clinical features like hematuria, Difficulty in micturition,irritation during micturition , are not correlated with the microscopic features including invasive and non invasive urothelial carcinoma.

A Spearman’s analysis was done to compare Her 2-neu expression with tumour grade. Comparison between these two features was statistically significant with a  $p < 0.05$ . From this comparison we came to a conclusion that Her 2- neu overexpression correlates well with the grade of the tumour.

Table 2: Comparison of HER2 status (n = 117)

Tumour Grade	HER2 protein overexpression NO. (% of cases)	
	Absent	Present
Low	24 (57.2)	18 (42.8)
High	15 (20)	60 (80)



**Fig.1:** HER2 expression in urothelial carcinoma. **A** HER2 positive staining scored as 3+.

**B** HER2 moderate staining scored as 2+. **C** HER2 negative staining scored as 1+

### Discussion

Urothelial carcinoma bladder is the fourth most prevalent type of cancer in men worldwide with male female ratio of 3 to 4:1<sup>1</sup>. Most patients with invasive urothelial carcinoma have atleast microscopic hematuria. The most common clinical symptom is painless gross hematuria, followed by urgency, nocturia and dysuria. Patients with diffuse tumours most commonly presents with irritative urinary symptoms. Late presentation with a large tumour mass may cause urinary obstructive symptoms.<sup>2</sup> Outcomes can be different in patients at the same pathological stage or grade. HER2 is a potential therapeutic target and one of the most frequently amplified oncogenes in bladder cancer. HER2/neu overexpression was investigated in a large number of

malignant tumors: breast, prostate, urinary bladder carcinomas.<sup>3,4,5</sup> In the case of the urothelial carcinomas of the urinary bladder, HER2/neu expression is variable, with an incidence between 2 and 74%<sup>6,7,8</sup>. HER2 presents a potential prognostic factor and may lead to HER2 targeted therapy to offer a survival advantage in patients with Urothelial Carcinoma.

Most papers have shown that HER2 overexpression was significantly correlated with poor clinicopathological factors and poor outcome in urothelial carcinoma

Gholamreza Pourmand and et al her2/neu overexpression as a prognostic factor in urothelial carcinoma of the bladder: a comparative analysis resulted in Overall HER2 expression was detected in 31% patients. HER2 overexpression [staining intensity score (SIS)  $\geq$  2+] was observed in 32.5% cases; however, none of the controls showed HER2 overexpression.<sup>9</sup>

A study by Skagias L and et al showed HER 2/neu protein overexpression in 41/80 patients (51.25%) demonstrating an increase in expression rate corresponding to progressively advanced tumour stage and tumour grade.<sup>10</sup>

In the present study HER2 overexpression was seen in 80 % of High Grade tumour and only

42.8 % in low grade tumour .Thus we noticed a trend of increased HER2 expression with increase tumour grade.

Also we notice that the clinical features does not correlate with the grade of the tumour.

### Conclusion

The role of HER2 status on urothelial carcinoma prognosis is still unclear. The overexpression of HER2 protein is noted in patients who appeared to have a more aggressive disease and therefore merits consideration. From this study we came to a conclusion that Her 2- neu overexpression was seen more significantly in High Grade tumours.(p < 0.05) So, there is enough evidence that supports the introduction of trastuzumab as an adjuvant therapeutic agent in the cases with HER2/neu overexpression. But a further larger and prospective studies will be needed to ascertain the frequency of HER2 alteration and the role of HER2 in the aggressive behavior.

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