Histomorphological Study of Urinary Bladder Tumor and Status of HER2/Neu and Ki67 Expression in Urothelial Carcinoma

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Worldwide proliferation marker Kinase inhibitor Ki67 and Human epidermal growth factor receptor2(HER2/neu) both are focused as more reliable biomarker for the risk of prognosis and also useful for targeted therapies for urinary bladder tumor. The present study has used the 2004 WHO grading system of urothelial carcinoma and the AJCC/UICC T staging system of the urothelial carcinoma of the bladder. To observed the status of Ki67 and HER2/neu in uroepithelium as compared with different stages and grades of urothelial carcinoma with special emphasis on low grade and high grade lesions to reveal their help as an ancillary technique in the diagnosis a cross sectional study was conducted in the Department of Pathology, Dhaka Medical College, Dhaka from January 2016 to December 2017 with 50 patients with urothelial carcinoma attending in Department of Urology, Dhaka Medical College Hospital, Dhaka. HER2/neu and Ki 67 IHC were assessed and compared by chi-square (x²) tests, unpaired student’s ‘T’ test or ANVOA test with p value <0.05 at 95% CI considered as significant. The mean age was 60.9±13.1 years old and the male to female ratio were 4:1. Among the histological variety, 100% of our patient showed urothelial carcinoma with significant male preponderance. A total of 72% of the patients had high grade and 28% had low grade urothelial carcinoma. A total of 100% of the patients presented with painless hematuria. Among 50 patients 68.0% had tumor level of extension up to PT1(sub epithelial connective tissue) and 32% up to PT2(muscularis propria) in their biopsy specimen. The incidence of smoking was much higher (72%) among patients with high grade urothelial carcinoma. Immunohistochemical expression of Her2/neu and Ki 67 revealed that there was no significant correlation between the expression of these markers with the age and gender (P value >0.05). There was significant association between the expression of Her2/neu (p value <0.030) and Ki67(P value <0.03) with the 2004 WHO grading system of urothelial carcinoma. Ki 67 and HER2/neu expression association with tumor grading can help in predicting the appropriate clinical outcome and selecting patients who may benefit by targeted therapy and avoid over treatment.


Key words: Bladder cancer; gender; smoking; transitional cell carcinoma; HER2/neu; Ki67

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Intruduction

Urinary bladder carcinoma is one of the most common cancer of genitourinary system. It is the 4th commonest cancer in man and 8th in women in the world with M: F ratio of 3:1.\(^1\)\(^2\) About 95% of the bladder tumors are of epithelial origin. Though the prevalence is more in developed countries, but now incidence is gradually increasing more in developing countries like Bangladesh, India etc due to industrialization and smoking habit. In our country bladder cancer also seems to be increasing due to increase number of aging people, expose to carcinogen and improved facilities of investigations. In Bangladesh a few populations based studies are available regarding the prevalence.\(^3\)

The urothelial carcinoma represents about 90-95% of all urinary bladder tumor. Histological tumor grading and staging are known prognostic factors for bladder cancer. The accurate prognosis with any single factor is difficult to predict. There are literature data of numerous studies demonstrating the therapeutic and prognostic value of biomarkers involved in the biomolecular mechanism of urothelial carcinoma.\(^4\) Considerable attentions has been given to the identification of prognostic biomarkers of urinary bladder carcinoma.\(^5\) Therefore, the prime interest is being currently focused on protein and genetic markers as they may become therapeutic target. The therapeutic weapons are limited in UC and they permit only a limited improvement.\(^6\)\(^7\)

HER2/neu and Ki67 both are currently focused as more reliable prognostic factors to assess accurate prognosis and useful therapies. HER2/neu is a glycoprotein similar to EGFR family that has tyrosine kinase activity. It acts as an oncogene.\(^8\)\(^9\)\(^10\) Most of the studies on HER2/neu have been carried out in breast cancer. It has now been recognized in other forms of cancers such as colon, bladder, ovarian, uterine endometrial carcinoma, stomach and esophagus carcinoma. Its over expression seems to be correlated with recurrence, higher grade and worse prognosis.\(^11\) Ki67 is a non-histone nuclear protein, known to be strictly associated with cell proliferation.\(^12\)\(^13\) It established as an independent predictor of recurrence, progression and response to immunotherapy. Different studies observed that Ki67 proliferation index has increased in high grade carcinoma with or without invasion.\(^14\)

Methods

A cross sectional study was conducted in the Department of Pathology, Dhaka Medical College, Dhaka from January 2016 to December 2017 with 50 patients with urothelial carcinoma attending in Department of Urology, Dhaka Medical College Hospital (DMCH), Dhaka. Clinically suspected patient (both male and female) were admitted as new cases of bladder tumor at Urology Department, DMCH with clinical symptoms like macroscopic hematuria, dysuria etc. Patients with these complain were advised for radiological examination. About 73 patients were reported as bladder tumor by the radiologists and were subject to do cystoscopy and transurethral resection (TUR) or biopsies of the suspicious mass.

A total of 50 histologically diagnosed urinary bladder tumor cases were selected from 73 radiologically and clinically suspected bladder tumor cases. Twenty three samples were excluded for tissue necrosis, inadequacy and cautery effect. During the collection of specimen, all relevant information were recorded systematically in a prepared proforma. All the cases were numbered chronologically and the same number was given to histological as well as in immunohistochemical slides. Bladder tumors
were sampled or removed with biopsy instrument. All obtained specimens were immersed in 10% buffered formalin. These samples were fixed for 6 hours to 48 hours which was required for proper H&E and immunostaining. Under fixation may cause false IHC result. HER2/neu and Ki 67 IHC were assessed and compared by chi-square ($x^2$) tests, unpaired student's ‘T’ test or ANVOA test with p value $<0.05$ at 95% CI considered as significant.

In this present study, the evaluation of HER2 was generally carry out using the American Society of Clinical Oncology/College of American Pathologists guideline for breast cancer.$^{15,16}$ This guideline has been updated in 2013.

Assessment of Ki67 Immunohistochemical Staining done according to Jawad, Ali and Kamal (2016)$^{17}$ and performed qualitatively by counting the percentage of positive cells (labeling index, LI) out of the total number tumor cells was calculated. Only distinct immune reactive tumor cell nuclei were counted.

**Result**

Table I: Demographic profile of the patients (n=50)

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\leq$60</td>
<td>29</td>
<td>58.0</td>
</tr>
<tr>
<td>&gt;60</td>
<td>21</td>
<td>42.0</td>
</tr>
<tr>
<td>Mean ± SD (Min-Max)</td>
<td>$60.9 \pm 13.1$ (20 - 88)</td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td><strong>Socioeconomic status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>19</td>
<td>38.0</td>
</tr>
<tr>
<td>Low</td>
<td>31</td>
<td>62.0</td>
</tr>
<tr>
<td><strong>Personal history</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>36</td>
<td>72.0</td>
</tr>
<tr>
<td>Betel nut chewing</td>
<td>36</td>
<td>72.0</td>
</tr>
</tbody>
</table>

Table I shows demographic profile of the patients.
Table II: Distribution of patients according to smoking habit (n=50)

<table>
<thead>
<tr>
<th>Smoker</th>
<th>Low grade (n=14)</th>
<th>High grade (n=36)</th>
<th>Total</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>11 (78.6)</td>
<td>25 (69.4)</td>
<td>36 (72.0)</td>
<td>0.517</td>
</tr>
<tr>
<td>No</td>
<td>3 (21.4)</td>
<td>11 (30.6)</td>
<td>14 (28.0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14 (100.0)</td>
<td>36 (100.0)</td>
<td>50 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

Fisher’s Exact test was done to measure the level of significance. Figures in the parenthesis denote corresponding %.
Table II shows there was no significant difference in smoking habit between low and high grade tumor.

Figure 1. Pie chart showing the tumor grading of study patients

Table III: Distribution of patients according to HER2/neu score in low and high grade tumor (n=50)

<table>
<thead>
<tr>
<th>Grading</th>
<th>Her2 expression</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Equivocal</td>
</tr>
<tr>
<td>Low grade</td>
<td>13 (92.9)</td>
<td>1 (7.1)</td>
</tr>
<tr>
<td>High grade</td>
<td>15 (41.7)</td>
<td>11 (30.6)</td>
</tr>
</tbody>
</table>

Fisher’s Exact test was done to measure the level of significance, s= significant
Figures in the parenthesis denote corresponding %.
Table III shows that positive value of HER2/neu expression is significantly higher in high grade tumor (P<0.05).

Table IV: Distribution of patients according to Ki-67 expression in low and high grade tumor (n=50)

<table>
<thead>
<tr>
<th>Grading</th>
<th>Ki 67</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Low grade</td>
<td>7 (50.0)</td>
<td>7 (50.0)</td>
</tr>
<tr>
<td>High grade</td>
<td>29 (80.6)</td>
<td>7 (19.4)</td>
</tr>
</tbody>
</table>

Chi-square test was done to measure the level of significance. s=significant
Figures in the parenthesis denote corresponding %.

Table IV shows Ki-67 expression was found more in high grade carcinoma then low grade carcinoma. The difference is statistically significant (p=0.031)

Table V: Distribution of the study patients according to grading with Her2/neu and Ki67 (n=50)

<table>
<thead>
<tr>
<th>Grade</th>
<th>HER2 (+ve)</th>
<th>HER2 (-ve)</th>
<th>HER2 (Equivocal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ki67(+ve)</td>
<td>Ki67(-ve)</td>
<td>Ki67(+ve)</td>
</tr>
<tr>
<td>Low grade</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>6 (37.5)</td>
</tr>
<tr>
<td>High grade</td>
<td>10 (100.0)</td>
<td>0 (0.0)</td>
<td>10 (62.5)</td>
</tr>
</tbody>
</table>

Figures in the parenthesis denote corresponding %.

Table V shows HER2/neu and Ki67 expression according to histologic tumor grading
Figure 2. Photomicrography showing high grade urothelial carcinoma. (Case No: 45, H&E x400)

Figure 3. Photomicrography showing positive (score 3+) membrane reactivity of HER2 protein in high grade urothelial carcinoma. (Case No:45, IHC for HER2 x 400)

Figure 4. Photomicrography showing positive of Ki67 in high grade urothelial carcinoma (Case No:45, IHC for Ki67 x 400)

Figure 5. Photomicrography showing low grade urothelial carcinoma. (Case No: 40, H&E x400)

Figure 6. Photomicrography showing no membrane reactivity of HER2 protein in low grade urothelial carcinoma. (Case No: 40, IHC for HER2x400)

Figure 7. Photomicrography showing negative Ki67 in low grade urothelial carcinoma. (Case No:40, IHC for Ki67 x400)
Figure 8. Photomicrography showing high grade urothelial carcinoma. (Case No: 12, H&E x400)

Figure 9. Photomicrography showing no membrane reactivity of HER2 protein in high grade urothelial carcinoma (Case No: 12, IHC for HER2x400)

Figure 10. Photomicrography showing positive Ki67 in high grade urothelial carcinoma. (Case No: 12, IHC for Ki67x400)

Discussion

In this study, maximum (58.0%) patients were below or equal to 60 years and 42.0% patients were more than 60 years old. mean age of the patients was 60.9 ± 13.1 and is similar to the study of Jawad, Ali and Kamal (2016) which was 58.72±1.6. In this study male to female ratio was found 4:1. Similar findings were also stated in the study of Jawad, Ali and Kamal, (2016). Male were predominant and This study shows the incidence of tumor is more in male (80.0%) than female (20.0%). Male to female ratio was found 4:1.

In our present study it was observed that majority (62.0%) of the patients came from low and 38.0% from middle socio economic condition and involved in different occupation. Two third of them were cultivator and only one patient worked in dye factory. Not only rapid industrialization and urbanization but also excess use of insecticide and fertilizer of the subcontinent particularly our country for the last few decades probably playing an important role for increasing incidence of UBC. Similar observation was also made by Kibria et al. (1997) in Bangladesh.

Maximum patients had habit of smoking and betel nut chewing. In this present study 72% patients had habit of smoking and betel nut chewing. Most of the male patients in this study had habit of both cigarette and betel nut and females had habit of betel leaf with betel nuts. Chinnasamy et al. (2016) revealed most of bladder cancer patients (71.2%) had smoking habit which was consistent with this study result. Chou et al. (2013) found 24.9% of urothelial cancer patients had smoking habit.

The histologic cell type of bladder cancer is geographically different. In our subcontinent urothelial carcinoma is the most common type. In our study, all the 50 (100%) cases were histologically transitional cell carcinoma. An Indian study in Kashmir by Jeelani et al. (2004) reported 98% TCC and 2% adenocarcinoma. A related study
conducted by the Urology Department of BSMMU in 1088 patients of ten different hospital of Dhaka city, observed 96.7% TCC, 1.2% squamous cell carcinoma, 1.6% adenocarcinoma and 0.5% other type of urinary bladder cancer (Hossain, 2011). In another study in Egypt by Shawky (2013) reported 43.8% squamous cell carcinoma followed by 40.6% TCC. Jemal et al. (2008) found that the endemic infection with Schistosoma species in Africa and Egypt was responsible for squamous metaplasia and subsequently squamous cell carcinoma in urinary bladder.

The patients in this study were grouped according to WHO grading of urinary bladder carcinoma. It was observed that 36 (72.0%) patients had high grade urothelial carcinoma (HGUC) and 14 (28.0%) patients had low grade urothelial carcinoma (LGUC). Chou et al., (2013) in their study found 56.8% high grade and 43.2% low grade tumor. Incidence of high grade UC patient was more in our study. In our country the probable cause may be poor economic condition, lack of knowledge, lack of urological facilities as well as social and religious restrictions especially for female patients which prevent them from utilizing hospital facilities.

The HER2/neu acts as an oncogene. HER2 expression was evaluated by immunohistochemistry in 50 cases of our study. Of the total 36 high grade Urothelial Carcinoma, HER2/neu expression was found (score3+) in 27.8% cases, equivocal (score 2+) in (30.6%) cases and rest (41.7%) were negative (score 0 & score 1+). No positive HER2/neu was observed in low grade. All the HER2/neu positive cases were found only in high grade cases but no positive expression was seen in low grade tumor.

Ki67 expression in high and low grade UC were 80.6% and 50% respectively. 7/36 (19.4%) showed negative expression of Ki67 in high grade UC. 7/14 (50%) low grade tumor showed positive expression of Ki67. Most of the positive Ki67 expression cases were found in high grade tumor.

10(100%) morphologically high grade tumor present score 3+ HER2/neu with positive Ki67 expression. 11(30.6%) high grade tumor showed equivocal expression of HER2/neu of which 90% showed Ki67 positivity. 15 morphologically high grade tumor expressed Her2/neu negativity with 10(62.5%) positive Ki67 expression.

All Her2/neu positive cases were also Ki67 positive. Among the 12 HER2/neu equivocal cases, 09 show positive Ki67 expression. Ki67 also shows positive expression in both high and low grade UC that were HER2/neu negative. So to find out the accurate prognosis, Ki67 expression in low grade and HER2/neu in high grade must get proper attention.

Significant correlation was observed with different grading of UC according to 2014 grading system. Co expression of HER2/neu and Ki67 were observed in 10/36 high grade urothelial carcinoma which are aggressive in nature.

HER2/neu positive tumors can be benefited by Herceptin therapy. Low grade tumor with negative HER2/neu but high Ki67 may need more aggressive therapy. So to find out the accurate prognosis, Ki67 expression in low grade and HER2/neu in high grade must get proper attention. HER2/neu and Ki67 overexpression have a relationship with the grading of urothelial carcinoma and can be used to assess controversial cases. They can help us to estimate the accurate biological behavior of urothelial carcinoma to select the appropriate treatment protocol.
The expression profile of both biomarkers may be useful for selecting high risk patients with bladder cancer for proper treatment. Hence patients who have a low risk of recurrence, need to identify in order to avoid over treatment as well as those who are likely to progress in order to treat them more aggressively. In Bangladesh no study was conducted on both HER2/neu and Ki67 expression and association in urothelial carcinoma. This study could have been more effective if more number of urothelial carcinoma cases were included and follow up was done to see the progression of the disease and recurrence.

**Limitations**

Reliability and reproducibility of IHC technique was a major limitation. Fluorescent in situ hybridization (FISH) could not be done for the equivocal cases due to financial limitation.

**Recommendation**

Use of immunohistochemistry in urinary bladder carcinoma for the screening of high risk patients.

Second confirmatory test with FISH for equivocal cases in IHC.

Further study with more sample size with cystectomy specimen and with follow up.

**References**


