Journal of Research in Applied and Basic Medical Sciences 2023; 9(1): 11-23

Original Article

RABMS Journal of Research in Applied and Basic Medical Sciences

Expression of *Her2/neu* in Urothelial Neoplasms and its Association with Histopathological Prognostic Parameters

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Abstract

***Background & Aims*:** Bladder carcinoma is the second common malignancy of the urogenital system. Bladder malignancy encompasses 5.2% of all forms of cancer. According to study, *Her2/neu* expression can be considered as a prognostic clinical biomarker for bladder cancer. Target therapy using novel and recombinant chemodrugs such as transtuzumab can be applied in *Her2/neu* positive cases. Current study aimed to evaluate urothelial neoplasms incidence, to assess *Her2/neu* expression, and to compare its expression with prognostic factors.

***Materials & Methods*:** The present study is a prospective study conducted in the Department of Pathology, Narayana Medical College and General Hospital, Nellore, India, for a period of 2 years from June 2019 to June 2021. All the urothelial neoplasm cases reported during the study period were included, and *Her2/neu* immunohistochemistry has been assessed for the cases.

***Results*:** Among 71 bladder specimens**,** 48 had urothelial carcinomas (54% of high grade and 46% of low grade), and 20 had benign neoplasms. Among high-grade carcinomas, 91% were muscle invasive and among low grade, 55% were non-muscle invasive. Lateral wall is the common site of urothelial carcinoma. The mean age for high-grade carcinomas was 61-70 years, and it was associated with higher grades and stages of the tumor. In high grade and low-grade carcinomas, males outnumbered females. A significant correlation observed between tumor grade and stage. A significant association was found between *Her2/neu* overexpression with the grade and size of the tumor. No association was found between *Her2/neu* expression and age, gender, stage, and invasion.

Conclusion: It is concluded that identifying the expression of *Her2/neu* in urothelial carcinoma can help identify eligible candidates for targeted therapy.

***Keywords*:** Transurethral Resection of Bladder Tumor, Hematoxylin, Eosin, Human Epidermal Growth Factor receptor 2

Received 18 July 2022; accepted for publication 03 December 2022

Introduction

Bladder cancer (also known as urothelial carcinoma) is a prevalent and aggressive malignancy among 0.6 million of new cancer cases, and causes 199,900 deaths annually globally (1). Urothelial neoplasms comprise non-invasive urothelial neoplasms and infiltrating urothelial carcinomas. The urinary bladder is the most common site for cancer development in the urinary tract (2, 3). The lateral wall of the urinary bladder is the commonest site for malignancy in the urinary tract, followed by posterior wall and trigone. In India, according to the recent reports of National Cancer Registry Programme, the overall incidence rate of the urinary bladder cancer is 2.25% (per 100,000 annually), 3.67% among males and 0.83% for females (4). Nearly 80% of the patients fall between 50 and 80 years of age with a male-to-female ratio of 3:1(5). Its development depends on a combination of genetic and environmental factors (6). Incidence of bladder tumors are high in industrial areas especially in those associated with petrochemicals and with exposure to cigarette smoke and arylamines (7). Painless hematuria is the most common presentation (8). The most important prognostic markers are tumor stage and grade (9). Predicting an accurate prognosis with a single factor is difficult, due to significant inter-observer variability in reporting the stage and grade (10). Therefore, role of protein and genetic markers as more reliable prognostic factors need to be studied (11). The *Her2/neu* oncogene encodes for tyrosine kinase transmembrane growth factor receptor. On immunohistochemistry (IHC), *Her2/neu* protein overexpression correlates with increased tumor grade in urothelial carcinoma (10, 11).

Most urothelial carcinomas express both *CK-7* & *CK-20* whereas primary adenocarcinoma of bladder only expresses *CK-20*. Urothelial differentiation markers uroplakin, *GATA-3*and *p63* aids in differentiating primary urothelial carcinoma from renal cell carcinoma, prostatic carcinoma, and nephrogenic adenoma.

The *Her2/neu* gene is located on chromosome 17q and encodes for a tyrosine kinase receptor, which is thought to control cell growth and development (12,13,14). Its activation increases the mitotic activity and metastatic potential of the cell, leading to oncogenic transformation. *Her2/neu* over expression and amplification was first identified in a human breast cancer cell line. It has been considered as a prognostic marker in breast carcinoma, particularly in lymph node-positive cases (15,16). The over expression of *Her2/neu* have been observed in various organs like stomach, colon, bladder, prostatic gland, salivary gland, ovary, and uterus. Since the important prognostic and therapeutic impact that *Her2/neu* status had in breast carcinoma, more interest has been given to its expression in other cancers (17). In 1990, Zhau et al. first reported an increased amplification and an over expression of *Her2/neu* in bladder cancer (19). Since then, several studies have been conducted and the over expression of *Her2/neu* in urothelial carcinoma ranged between 17% and 76% of invasive carcinoma (20).

The prognostic impact of *Her2/neu* on urothelial carcinoma is variable among several studies. Various studies found that *Her2/neu* over expression is predictive of bladder cancer death in patients with invasive cancer. Kolla et al. observed a significantly high disease-free survival in *Her2/neu* negative patients compared to *Her2/neu* positive patients; this difference was more profound in the patients with locally advanced disease (21).

An anti *Her2/neu* antibody was proposed as a therapeutic tool in invasive bladder cancer with response rate ranges from 3 to 63% (21). Thus, a reliable evaluation is needed to introduce targeted therapy in the management of invasive urothelial carcinoma.

In the current study, evaluation of *Her2/neu* expression in urothelial neoplasms is done and it is correlated with clinicopathological variables like age, gender, grade, stage, and invasiveness of tumor that might help in risk stratification and patient management. *Her2/neu* overexpression can be a potential to offer anti-*Her2/neu* therapies in locally advanced and metastatic disease.

In the present series, we evaluated the immunohistochemical expression of *Her2/neu* and correlated their expression with various

clinicopathological variables that might predict patient outcome and further management.

Materials & Methods

This study is a prospective study done over a period of two years from June 2019 to June 2021 in the Department of Pathology, Narayana Medical College and Hospital, Nellore, India.

During our study period, we received 16,294 specimens for histopathological examination. Of the total 16,294 specimens, 223 cases belong to urinary system. 71 cases of bladder specimens were received and most of them were clinically suspected to have bladder carcinoma. Out of 71 bladder specimens, radical cystectomy specimens accounted for 16 cases, and remaining 55 cases were transurethral resection of the bladder tumor (TURBT) specimens.

All 71 bladder specimens were subjected for histopathological examination. 51 cases had benign neoplasms and 20 cases showed features of benign neoplasms. Due to lack of appropriate complete data, study was limited to malignant neoplasms.

Inclusion criteria:

All Cystectomy and transurethral resection of bladder specimens that are histologically diagnosed as urothelial neoplasms irrespective of age, gender, and tumor grade and stage are included in the study.

Exclusion criteria:

Non-neoplastic lesions, inadequate samples, and autolysed samples are excluded from the study.

Method of data collection:

Detailed history of the cases regarding age, sex, personal history, symptoms, site, and type of the procedure done were obtained for all 71 cases reported during the study period from surgical pathology records. All transurethral resections of bladder specimens were processed entirely and representative sections were taken from radical cystectomy specimens for histopathological examination.

The following clinical and pathological parameters were evaluated: age, gender, tumor site, tumor size, tumor grade, tumor stage, and invasiveness of the tumor.

Urothelial carcinoma was graded as high grade and low grade based on architectural distortion as well as cytological and nuclear atypia. They were sub-classified further based on the invasiveness as muscle invasive and non-muscle invasive bladder carcinomas. Among 71 cases, equal proportion of high- and low-grade urothelial carcinomas were selected randomly and 22 cases of low grade and 22 cases of high-grade urothelial carcinoma were included. Out of 22 cases of high-grade carcinoma, 20 were muscle invasive and 2 were non-muscle invasive. Among 22 cases of low-grade urothelial carcinoma, 10 were muscle invasive and 12 were non-muscle invasive carcinomas. These 44 cases were analyzed for immunohistochemical expression of *Her2/neu*.

Immunohistochemical Evaluation:

Immunohistochemical analysis of *Her2/neu* was performed in paraffin embedded tissue samples using supersensitive polymer HRP system based on non-biotin polymeric technology. 4-micron sections were cut from formalin fixed paraffin embedded tissue samples and transferred onto positively charged slides. Heat induced antigen retrieval was done. The antigen was bound with rabbit monoclonal antibody against *Her2/neu* and then detected by adding secondary antibody conjugated with horse-radish peroxidase-polymer and diaminobenzidine substrate.

Antigen	Vendor	Species	Dilution	Positive control
Her 2 neu	Pathnsitu	Rabbit	Ready to use	Breast carcinoma

Interpretation and scoring system:

The antibody treated slides were analyzed for the presence or absence of reaction, localization of the staining pattern, percentage of cells stained, and intensity of the reaction.

Evaluation of *her 2/neu* staining:

For assessing *Her2/neu* positivity, ASCO scoring system was used. According to this system, only membranous staining pattern was considered positive, and the level of *Her2/neu* expression was assessed semi-quantitatively by the intensity & percentage of cells stained and scored on a scale of 0-3+. A cytoplasmic staining was considered nonspecific.

Assessment of *Her2/neu* staining is done by taking score 0 and score 1+ as negative, score 2+ as equivocal, and score 3+ as strong positive.

Statistical analysis:

The statistical evaluation was performed by IBM-SPSS statistical package version 20. An initial analysis of collected variables was performed. Immunohistochemical expression of *Her2/neu* was analyzed and correlated with clinical variables like age, gender, and size as well as pathological variables like histologic grade, stage, and invasiveness of the tumor. Pearson's Chi-square test was used in analyzing these variables. In the present study, the P values below 0.05 were considered as significant.

Results

During the study period of 24 months from June 2019-June 2021, a total of 16,294 specimens were received in the Department of Pathology, Narayana Medical College and Hospital for histopathological examination. Of the total cases, 71 bladder specimens were received and this included both transurethral resection of bladder tumors (55 specimens) and radical cystectomy (16 specimens). Among 71 cases, 48 cases had reported as urothelial carcinoma (26 High grade and 22 low grade), 20 had benign neoplasms and 3 had other variants of bladder malignancy.

Among 26 cases of high-grade carcinoma (54%), 85% constituted muscle invasive (22 cases) and 15% were non-muscle invasive (4 cases). Out of 122 cases of low-grade carcinoma (46%), 45% were muscle invasive (10 cases) and 55% were non-muscle invasive (12 cases). Thus, we inferred from this study that the incidence of high-grade urothelial carcinomas outnumbered low-grade carcinoma and most high-grade carcinomas were muscle invasive and low-grade carcinomas were non-muscle invasive. Muscle invasion was more frequently encountered in high-grade than in low-grade carcinomas.

Other histologic variants like urothelial carcinoma with sarcomatoid differentiation, primary squamous cell carcinoma of the bladder have also been reported in our research.

Among 48 cases of urothelial carcinoma, 44 cases were selected based on availability of tissue block and clinical data. Equal proportions of high grade and low-grade carcinomas were taken into account for easy comparison. However, the frequency with muscle invasion was more in high-grade than in low-grade carcinomas. Thus, an equal number of muscle invasive and non-muscle invasive tumors were not taken into account.

Among 44 cases in the study population, 50% had high grade (22 cases) and 50% had low-grade (22 cases) urothelial carcinoma. About 91% of the high-grade carcinomas were muscle invasive and 55% of the low-grade carcinomas were non-muscle invasive.

In the study population, 77% were TURBT specimens (34 cases) and remaining 23% were resected specimen (10 cases). TURBT was performed more frequently than bladder resection because most patients present with advanced stage and thus palliative treatment was the main modality of the treatment. As bladder carcinoma was more common in older age group, the morbidity and mortality related to surgery were high and thus TURBT was performed more frequently.

Table 1. Age wise distribution of cases

Age group	High-grade carcinomas	Low-grade carcinomas
30-40	0	5
41-50	1	2
51-60	7	5
61-70	10	6
>70	4	4
Gender		
Male	19	15
Female	3	7
Size of the tumor		
1.5 -3 cm	4	6
3 – 5 cm	11	7
>5 cm	7	9

In this study, the peak incidence of urothelial carcinoma occurred above 60 years of age. The mean age group for high-grade carcinoma was 61-70 years and for low-grade carcinoma was 51-60 years. It is inferred that as age advances, the tumor grade is higher. It was clear that as age advances, the stage of the tumor was high and might affect the outcome of the patient.

Gender wise correlation of cases:

In the present series, males constituted 77% (34 cases) and females accounted for 23% (10 cases) of the urothelial carcinoma. Among high-grade carcinoma, males constituted 86% (19 cases) and females accounted for 14% (3 cases) of the cases. In low-grade carcinoma, males constituted 68 % (15 cases) and females accounted for 32% (7 cases). Thus, the overall incidence of urothelial carcinoma was higher in males than in females (Table 2).

Table 2: Showing gender-wise correlation of cases

Characteristic		Grade				Total	Statistical inference
		High invasion		Low invasion			
		MI	NMI	MI	NMI		
Sex	Female	3	0	3	4	10	<0.05
	Male	17	2	7	8	34	
	Subtotal	20	2	10	12	44	
Site wise correlation of bladder carcinoma							
Site of growth							
Anterior wall		1	0	0	0	1	
Anterolateral wall		1	0	0	0	1	
Lateral wall		10	1	10	8	29	
Lateral wall, base		1	0	0	1	2	0.515
Posterior wall		0	0	0	1	1	
Posterolateral wall		7	1	0	1	9	
Trigone		0	0	0	1	1	

Immunohistochemical expression of *Her2/neu*						
Her2/neu						
1+	2	1	5	6	14	Chi square=6.731, p value=0.035.
2+	15	1	3	6	25	
3+	3	0	2	0	5	
Tumor size						
1.5- 3.0 cm	3	1	0	6	10	0.0137
3.5- 5.0 cm	10	1	2	5	18	
>5.0 cm	7	0	8	5	20	
Tumor grade	High grade		Low grade			
Stage I	2		18		20	Chi square=23.467, p value=0.000
Stage II	17		4		21	
Stage III	3		0		3	

The most common site of urothelial carcinoma was lateral wall (29 cases) followed by posterolateral wall (9 cases). A statistical analysis between site of tumors and grade showed a *P*=0.515 without correlation (Table 2).

Size wise correlation of urothelial carcinoma:

For most high-grade tumors, size was 3-5 cm and for most low-grade urothelial carcinoma was more than 5 cm. In this study, as the size of tumor increased, the frequency with which the muscle invasion occurs also increased. Thus there exists a significant statistical correlation between tumor size and invasiveness (*P*=0.0137). However, no correlation was observed between size and the grade of the tumor (*P*= 0.08). The most of the stage I tumors were more than 5 cm in size. Stage II tumors were 3-5 cm range and stage III tumors were more than 5 cm in size. Thus, large size of the tumors are in higher stage. There is no significant correlation between size and stage of the tumor (*P*=0.121).

Correlation of tumor grade with stage:

From the chart below, most low-grade carcinomas were staged I and among 22 high-grade urothelial carcinomas, 17 were staged II tumor, 3 were stage III tumor, and 2 were staged I tumor. There was a statistical correlation between tumor stage and grade (Pearson's chi-square=23.467, *P*=0.000).

Immunohistochemical expression of *Her2/neu*:

The immunohistochemical expression of *Her2/neu* was evaluated by ASCO scoring and was scaled from 0 to 3+ score. For assessment of *Her2/neu*, score 0 and score 1+ are taken as negative, score 2+ taken as equivocal, score 3+ is taken as strong positive.

3 of all 22 high-grade urothelial carcinoma showed 3+ membranous positivity. 16 cases showed equivocal positivity which needs further *Her2/neu* demonstration by FISH. Remaining 3 high-grade urothelial carcinomas were negative for *Her2/neu* staining.

Among 22 low-grade urothelial carcinoma, 2 cases showed 3+ strong membranous positivity. 9 cases showed equivocal positivity, and the remaining 11 cases were negative for *Her2/neu* staining.

The inference from this study was that most low-grade urothelial carcinomas were negative for *Her2/neu* staining. *Her2/neu* staining among high-grade carcinomas were determined by various other factors which needs further research in the future. *Her2/neu* positive high-grade carcinoma patients can be considered for targeted therapy in the future (Pearson's chi-square=6.731, *P*=0.035).

Among 22 high-grade urothelial carcinoma, 3 (13.6%) were negative, 16 (72.7%) showed 2+ and remaining 3 (13.6%) showed strong 3+ membranous positivity. Out of 22 low-grade urothelial carcinoma, 11 (50%) were negative, 9 (40.9%) were 2+, and 2 (9.1%)

showed 3+ strong membranous positivity. There was a significant statistical correlation between *Her2/neu* expression and grade of the tumor.

Correlation *of Her2/neu expression with invasiveness:*

Among 30 invasive tumors, 7 were negative, 18 showed equivocal staining and 5 cases showed strong 3 + membranous positivity. Among 14 non-invasive tumors, 7 cases were negative, 7 were equivocal staining. There was no significant statistical correlation between *Her2/neu* expression and invasiveness of the tumor (Pearson's chi-square=7.834, *P*=0.098).

Table 3. Correlation of *Her2/neu* expression with gender, age, and tumor size

Variables	0 &1+	2+	3+	Statistical inference
Gender				
Male	10	22	2	Chi-square=5.866, P value=0.058.
Female	4	3	3	
Age				
30-40 yr	4	1	0	
41-50 yr	1	2	0	
51-60 yr	2	9	1	Chi-square=12.050, P value =0.149.
61-70 yr	5	8	3	
>70 yr	2	5	1	
Tumor size				
1.5- 3.0 cm	8	2	4	
3.5- 5.0 cm	1	16	8	Chi-square=22.366, P value=0.000.
>5.0 cm	0	1	4	

Correlation of *Her2/neu* expression with gender:

Among 34 male patients, 10 (%) patients showed negative staining, 22 (%) had equivocal expression and remaining 2 (%) showed strong 3+ membranous positivity. Out of 10 female patients, 4 (40%) showed negative staining, 3 (30%) showed equivocal staining, and 3 (30%) patients expressed 3+ membranous positivity. No significant statistical correlation was observed between *Her2/neu* expression and gender.

Correlation *of Her2/neu expression with age*:

In this study, age of the patient was divided into 5 groups starting from 30-40 years, 41-50, 51-60 years, 61-70 years, and above 70 years. Among 44 cases, strong 3+ membranous positivity was found in the 61-70 age group (3 cases) and one case each in 51-60 & above 70-year age group. There was no statistical correlation between *Her2/neu* expression and age of the patient.

Correlation *of Her2/neu expression with tumor size:*

It was evident that there was increasing expression of *Her2/neu* 3+ strong membranous positivity with increase in tumor size. Among 44 cases, stronh *Her2/neu* expression was found in the 3-5 cm range (1 case) and 4 cases with more than 5 cm tumor size showed strong expression. Thus, there was statistical correlation between *Her2/neu* expression and size of the tumor ($p<0.001$) (Table 3).

Correlation *of Her2/neu expression with tumor stage:*

Most stage I tumors showed negative and stage III tumors are equivocal staining, while stage II tumors exhibited variable expression (4 cases were negative, 14 cases showed equivocal expression, and 3 cases showed strong 3+ membranous positivity). There was no statistical correlation between *Her2/neu* expression and stage of the tumor.

From this study, we inferred that *Her2/neu* expression showed significant statistical correlation with the grade of the tumor and tumor size. Several other

variables like invasiveness, gender, age, and stage did not reveal significant statistical correlation with *Her2/neu* expression. Further research has to be implemented in the future to recognize the variables that affect the immunohistochemical expression of *Her2/neu* in bladder carcinoma.

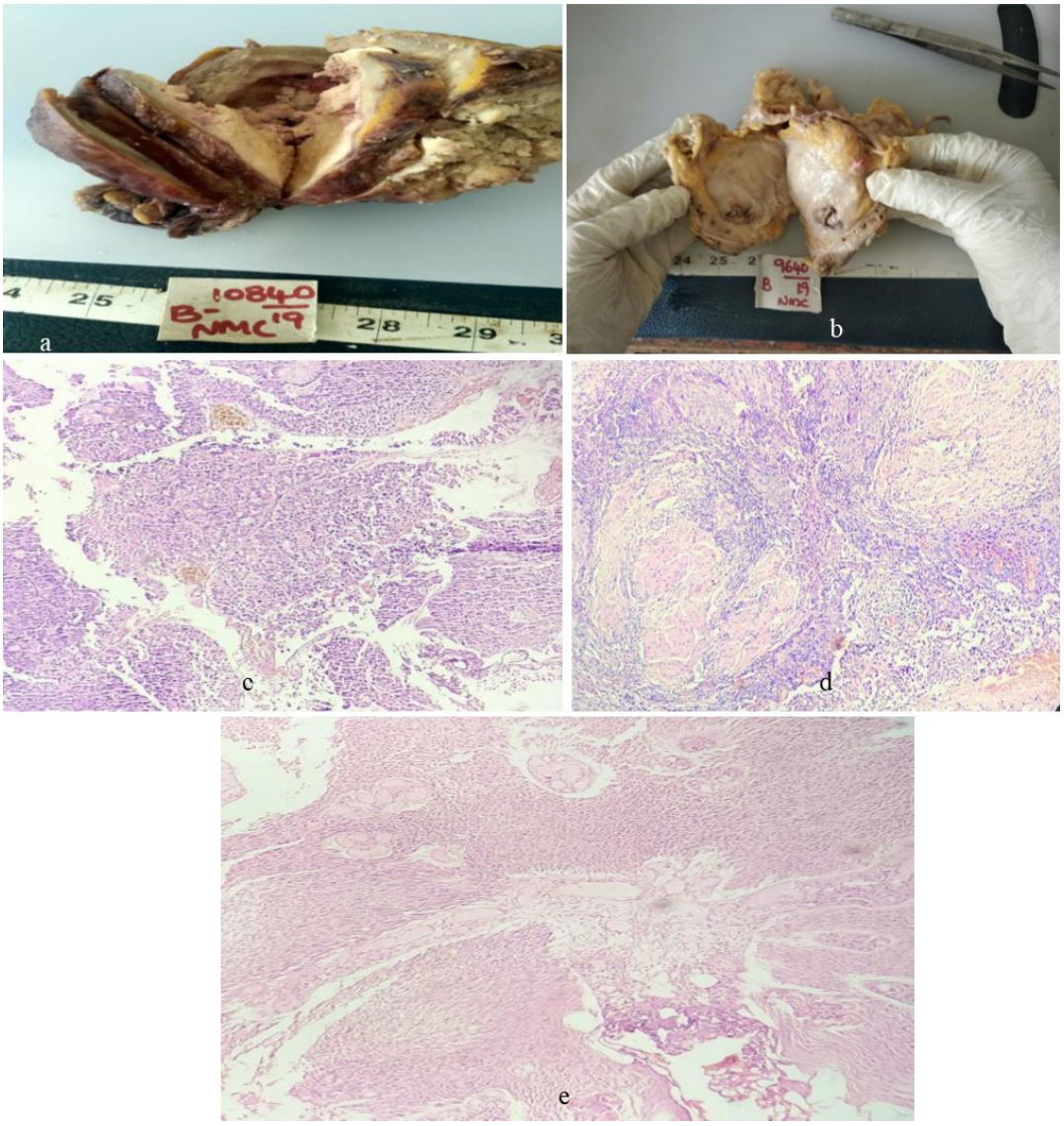

Fig. 1. H&E staining images. a: BX10840/19- radical cystectomy – cut surface. B: BX 9640/19 RADICAL CYSTECTOMY -CUT SECTION. c: HIGH Grade tumor in(H&E) 400 X. d: Tumor tissue showing muscle invasion (H&E, 40x). e: High-grade urothelial carcinoma (H&E) 100X.

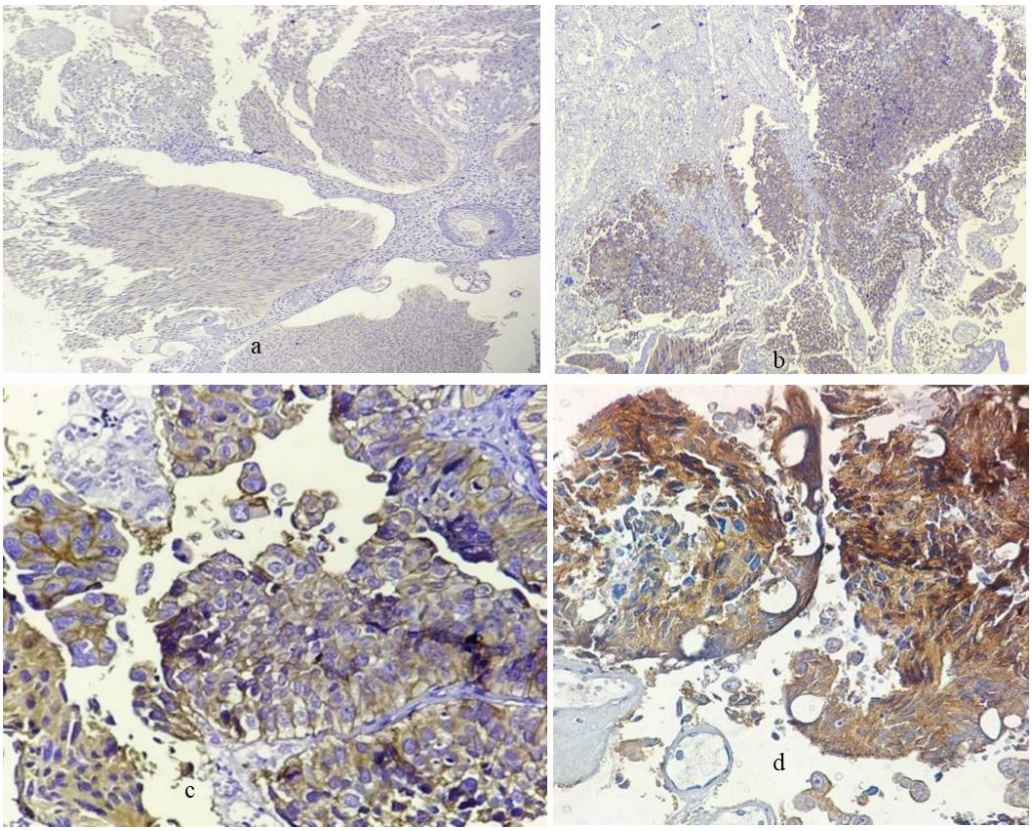

Fig. 2. H&E images. a: high-grade tumor showing equivocal Her2, 100 X. b:BX Tumor areas showing *Her2/neu* expression(IHC stain, 400x).c:2+ membranous staining of Her2-400X.d:Her2 showing 3+ strong positivity(400X).

Discussion

As per the Indian cancer registry data, it is the ninth most common cancer and is three times more common in men than in women (22). Various prognostic factors have influenced the outcome of patient with urothelial carcinoma. In this study, we evaluated the immunohistochemical expression of *Her2/neu* in muscle invasive and non-muscle invasive bladder tumors and correlated their expression with various clinicopathological variables like age, gender, tumor size, site, grade, stagem and invasiveness of the tumor.

We received 71 bladder specimens, and 48 were reported as urothelial carcinoma. The incidence of urothelial carcinoma in our institute was 68% (48 cases). Among 68% of the urothelial carcinoma, low-grade urothelial carcinomas constituted 46% (22 cases) and high-grade urothelial carcinomas accounted for 54% (26 cases) of the cases. Based on the invasive nature, further stratification of high- and low-grade urothelial carcinoma was made. Out of 26 cases of high-grade carcinoma (54%), 22 cases (85%) were muscle invasive and 4 cases (15%) were non-muscle invasive. Among 22 cases of low-grade carcinoma (46%), 10 cases (45%) were, muscle invasive and remaining 12 cases (55%) were non-muscle invasive tumors.

Several studies showed various incidences of muscle invasive and non-muscle invasive tumors. According to Gupta et al. (23), 561 bladder cancer patients were included in the study and only 26% of the patients had muscle invasive disease at the time of presentation. In most studies non-muscle invasive tumors were more frequently encountered than muscle invasive tumors (18%).

In our institute as tertiary referral center, high-grade urothelial carcinomas outnumbered the low-grade urothelial carcinomas. Among high-grade urothelial carcinomas, muscle invasive tumors were most frequently encountered than non-muscle invasive tumors, and most of the low-grade urothelial carcinoma were non-muscle invasive tumors.

Out of 48 cases of urothelial carcinomas, equal proportion of high grade (22 cases) and low grade (22 cases) carcinomas were selected for easy comparison. Among 22 cases of high-grade urothelial carcinomas, 20 cases (91%) were muscle invasive and 2 cases (9%) were non-muscle invasive tumors. Out of 22 cases of low-grade urothelial carcinomas, 10 (45%) were muscle invasive and 12 (55%) were non-muscle invasive. The discrepancy of invasiveness as muscle invasive and non-muscle invasive tumor occurred due to the high incidence of muscle invasive tumor among high grade and most of the low-grade urothelial carcinomas were non-muscle invasive, which correlates with Gupta et al.

Type of the Specimen Received:

Among 71 bladder specimens, 55 were TURBT specimens (77%) and remaining 16 (23%) were radical cystectomy specimens. In the study group consisting of 44 cases of urothelial carcinoma (100%), 34 (77%) were TURBT specimens and 10 (23%) were radical cystectomy specimens, which included palliative cystectomy and anterior pelvic exenteration specimens. In our institute, TURBT was performed more frequently than cystectomy.

In this study, the peak incidence of urothelial carcinoma was above 60 years of age. The most common age group for high-grade urothelial carcinoma was 61-70 years and for low-grade urothelial carcinoma was 51-60 years. The incidence of bladder carcinoma is low among young age group and as age advances, tumor grade gets higher, correlating with the results of Yuvaraja et al. (7).

In the patients more than 50 years of age, the invasive nature of the tumor is more in high-grade urothelial carcinoma than in low-grade urothelial carcinoma. The tumor grade correlates directly with age and as age advances, the tumor grade is higher correlating with Yuvaraja et al.

In this study, the highest number of stage I tumor (6 cases) occurred in 61-70 age group and the maximum number of stage II tumor (8 cases) was found in 61-70 years age group. The stage III tumor was found most in the patients above 60 years of age with the exception of one case falling in 51-60 age group. It is inferred that stage III tumors were found in higher age group.

Among 44 cases of urothelial carcinoma, 77% (34 cases) were males and females constituted 23% (10 cases) of them. In this study, the male:female ratio for bladder carcinoma was 3.4:1. According to the results of Mungan et al. (24), the incidence of urothelial cancer was 3 to 4 times more in males than females, and it is in par with the present study.

Out of 22 cases of high-grade urothelial carcinoma, 86% (19 cases) were male and 14% (3 cases) were female. Among 22 cases of low-grade urothelial carcinoma, males constituted 68% (15 cases) and females accounted for 32% (7 cases).

Among high- and low-grade urothelial carcinomas, males outnumbered females and it is evident that urothelial carcinoma is more common in males than in females, concordant with the results of Mungan et al. (24).

Site and Size-Wise Distribution of Cases:

In the present series, the most common site of urothelial carcinoma was lateral wall (29 cases), followed by posterolateral wall (9 cases). Other locations like anterior wall, base, and trigone were less commonly involved. According to literature, Urothelial carcinoma predominantly involves the lateral and posterior bladder wall, close to the ureteral orifice. Present study is concordant with it (24). In this study, the size of the tumor for most of high grade (11 cases) was in the range of 3-5 cm, and for most of the low-grade urothelial carcinoma (13 cases) was more than 5 cm. The most muscle invasive fall in tumor size of 3-5 cm, and for most of non-muscle invasive carcinoma was 1.5-3 cm. It is inferred that grade of tumor is independent of its size, whereas the invasiveness of the

tumor depends on the tumor size discordant with the results of Heney et al. (25).

Correlating the Size and Stage of the Tumor:

Out of stage I cases, 6 were of tumor size ranging between 1.5 to 3 cm, 5 cases were in the 3 to 5 cm range, and 9 cases showed tumor size falling in the range over 5 cm. Most of the stage II tumors (13 cases) have their range between 3 to 5 cm and most of the stage III tumors were over 5 cm size. According to Bostwick (26) tumor size influenced the stage of the tumor, but not progression to muscle invasiveness. In the present study, tumor size showed correlation with the invasiveness of the tumor but no correlation with stage and grade.

Correlation of tumor grade and stage:

In this study, 20 cases (45%) were in stage I tumor, 21 cases (48%) were in stage II and 3 cases (7%) were in stage III tumor. Out of 20 stage I tumors, 2 cases (10%) were in high grade and 18 cases (90%) were in low-grade urothelial carcinoma. Among 21 stage II tumors, 17 (81%) had high grade and remaining 4 (19%) had low-grade carcinoma. 3 cases (100%) of stage III tumors had high grade. In this study, at the time of presentation, most high-grade carcinomas presented as stage II &III tumors compared to low-grade urothelial carcinomas, which presented as stage I & II tumors. A significant statistical correlation was observed between tumor grade and stage ($P<0.0001$).

Immunohistochemical Expression of *Her2/neu*:

Immunohistochemical expression of *Her2/neu* was studied in 44 cases of urothelial carcinoma and its expression is compared with clinical variables like gender, age, size of the tumor, and pathological variables like histologic grade, invasiveness of the tumor, and stage of the tumor. Among 22 high-grade urothelial carcinoma, negative (score 0) to weak membranous positivity (score 1) was expressed in 3 cases (13.6%), 16 cases (72.7%) revealed equivocal staining pattern (score 2), and 3 cases (13.6%) expressed strong membranous positivity (score 3). Out 22 low-grade urothelial carcinoma, 11 cases (50%) showed negative to weak membranous positivity, 9 cases (40.9%) showed equivocal staining pattern and 2 cases (9.1%) showed strong membranous positivity.

Several other studies reported variable staining pattern ranging from 21.7% to 80%. It is evident that 14% of the high-grade urothelial carcinoma expressed strong membranous positivity (score 3), whereas 50% of the low-grade urothelial carcinoma did not express *Her2/neu* (27).

Of 30 muscle invasive tumors, 7 cases (23%) had negative to weak membranous positivity, 18 cases (60%) showed equivocal staining pattern and 5 cases (17%) expressed strong membranous positivity. Among 14 non-muscle invasive tumors, 7 cases (50%) exhibited negative to weak staining pattern, 7 cases (50%) showed equivocal staining pattern and no case showed strong membranous positivity.

It is inferred that 17% of invasive tumors showed strong membranous positivity whereas 50% of non-muscle invasive tumor did not express *Her2/neu.* There was no significant statistical correlation between *Her2/neu* over expression and invasiveness of the tumor.

In this study, the size of the tumor is grouped into 3 ranges: 1.5-3 cm, 3-5 cm, and more than 5 cm. Of 44 urothelial carcinoma cases, strong membranous positivity was expressed in 2 ranges: 3-5 cm (1 case) and more than 5 cm (4 cases).

It is clear that there is strong expression of *Her2/neu* as the size of tumor is increasing. In our study *Her2/neu* expression is dependent on tumor size (P=0.000). This is contrary to the results of Ismail NEH et al. (28).

Out of 20 cases of stage I tumors, only one case (5 %) expressed strong membranous positivity, 9 cases (45%) had equivocal staining pattern, and 10 cases (50%) revealed negative staining pattern. Among 21 cases of stage II tumors, 4 cases (19%) expressed negative staining pattern, 14 cases (67%) had equivocal staining pattern, and 3 cases (14%) showed strong membranous positivity. Out of 3 cases of stage III tumors, 2 cases (67%) showed equivocal and 1 case (33%) showed strong membranous positivity. It is observed that immunohistochemical expression of *Her2/neu* is independent of the stage of the tumor.

In the present series, results are similar with Charfi et al. (29) and Nedjadi et al. (30) on comparing the immunohistochemical expression of *Her2/neu* with various clinical and pathological variables, it is evident that there is significant statistical correlation between *Her2/neu* expression with grade of the tumor and with the tumor size. Its expression is not affected by invasiveness of the tumor, gender, age, and the stage of the tumor.

Conclusion

The incidence of urothelial carcinoma in our institute was 68%. The majority of patients are over 60 years of age and there was an overwhelming male preponderance of urothelial carcinoma. As many patients presented with advanced stage disease, the need for palliative treatment is increasing and because of the advanced age, the mortality associated with surgery also increases. This study helped in evaluating the new molecular markers involved in the pathogenesis of urothelial carcinoma. The immunohistochemical expression of *Her2/neu* is evaluated and correlated with various clinicopathological variables. *Her2/neu* overexpressing patients can be considered for targeted therapy and overexpression is associated with poorer prognosis.

Acknowledgments

None declared.

Conflict of interest

None declared.

Funding/support

The Narayana Medical College and Hospital, Nellore, India financially supported this study.

Ethical statement

This study was conducted with the approval of the institutional ethics committee and the informed consent of the patients who provided the bladder specimens for histopathological examination.

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