Original Article

Immunohistochemical Expression of Ki67 and HER2/neu in Urothelial Tumors

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Abstract

Background: Urinary bladder cancer is the most common malignancy involving the urinary system and the ninth most common malignancy worldwide. Proliferation marker kinase inhibitor (Ki67) is a nuclear and nucleolar non-histone DNA-binding protein that is encoded by the MKi67 gene in humans. Human epidermal growth factor receptor 2 (HER2) is a transmembrane tyrosine kinase receptor that is involved in cellular growth, survival, and migration.

Materials and Methods: The cross-sectional study was conducted for the duration of one year in the Department of Pathology, BPS Medical College for Women, Khanpur Kalan, Sonepat. Tissue sections, after proper processing, were stained with haematoxylin and eosin. A total of 50 histopathologically proven cases of urothelial tumors were included in the study. Correlation of urothelial tumors was studied with age, gender, smoking, and tumor grading. Urothelial tumors were classified according to the WHO classification of tumors of the urinary bladder (2016), and IHC markers Ki67 and HER2/neu expressions were evaluated.

Results: Among 50 cases of urothelial tumors, 25 (50%) cases were low-grade urothelial carcinoma, followed by 22 (44%) cases of high-grade urothelial carcinoma. Two cases of squamous cell carcinoma and one case of papillary urothelial neoplasm of low malignant potential were also observed. Ki67 expression was significantly associated with urothelial carcinoma (p-value = 0.01). HER2/neu expression showed a significant association with muscle-invasive tumors (p-value = 0.02).

Conclusion: Positive Ki67 and HER2/neu expression correlated with tumor grade. Assessing HER2/neu status could identify patients with high-grade disease who might benefit from adjuvant HER2/neu treatment after radical cystectomy.

Keywords:

Urothelial tumors, LGUC, HGUC, squamous cell carcinoma, PUNLMP, Ki67, HER2/neu.

Introduction

Urinary bladder cancer is the most common malignancy involving the urinary system and the ninth most common malignancy worldwide. According to Indian Cancer Registry data, in men, it is the ninth most common cancer, accounting for 3.9% of all cancer cases in India. Men are affected three to four times more often than women [1,2]. The major prognostic factors in bladder

carcinoma are the depth of invasion into the bladder wall and the degree of differentiation of the tumor on light microscopy. Other factors include age of the patient, tumor grade, stage, and lymph node status [3].

According to the 2016 WHO classification, types of urinary bladder tumors are urothelial carcinoma, squamous cell carcinoma, glandular neoplasms, urachal carcinoma, tumors of Müllerian type, neuroendocrine tumors, melanocytic tumors, and mesenchymal tumors. Urothelial carcinoma includes infiltrating urothelial carcinoma, non-invasive urothelial lesions, and variants such as nested, microcystic, micropapillary, plasmacytoid, sarcomatoid, giant cell, lipid-rich, and clear cell. These are classified into low, intermediate, and high grade on histopathological examination, and further classified in accordance with muscle invasion as non-muscle invasive and muscle invasive [4].

The treatment of urothelial carcinoma is largely based on histological grade and stage [5]. The primary therapy for invasive papillary tumors is transurethral resection of the bladder tumor (TURBT). For low-grade tumors, a single intravesical treatment with mitomycin, doxorubicin, or epirubicin may be added; for high-grade tumors, intravesical Bacillus Calmette–Guérin (BCG) is the treatment of choice. Urothelial carcinoma in situ is managed as for high-grade urothelial carcinoma. T1 tumors are generally treated similarly, often with a repeat transurethral resection to rule out muscularis propria invasion [6].

Immunohistochemistry (IHC) is a method for locating specific antigens in tissues based on antigen–antibody recognition. It uses specific antibody–antigen binding for visualization under a light microscope. Immunohistochemistry determines the proliferative activity of tumors by assessing the expression of various biomarkers in tissues and is used as an aid in the differential diagnosis and classification of cancer, microscopic tumor staging, prognosis, and prediction of response to specific therapy [7].

Proliferation marker kinase inhibitor (Ki67) is a nuclear and nucleolar non-histone DNA-binding protein that is encoded by the MKi67 gene in humans. Ki67 is a cellular marker for proliferation, present during the active phases of the cell cycle (G1, S, G2, and mitosis), but absent from resting cells. High Ki67 proliferation indices have been documented in both invasive and non-invasive high-grade bladder carcinomas [8].

Human epidermal growth factor receptor 2 (HER2) is a transmembrane tyrosine kinase receptor that is involved in cellular growth, survival, and migration. Its coding gene is located on chromosomal band 17q21, a known proto-oncogene (erb2). HER2 overexpression is associated with poor cancer prognosis, and anti-HER2/neu therapy is well established for cancers with HER2/neu overexpression [8].

This study aims to investigate the expression of Ki67 and HER2/neu in urothelial tumors and to correlate it with histopathological grade and clinical stage.

Materials and Methods

Fifty histologically proven cases of urothelial tumors in the duration of one year (from September 2022 to September 2023) were included in this cross-sectional study after getting approval from the ethical committee. The clinical data of the cases were recorded, and blocks were retrieved from the records. Sections were cut and stained with hematoxylin and eosin for histopathological diagnosis, tumor grade, and tumor staging. We did Ki-67 and Her2neu immunohistochemical staining using serial sections from the paraffin-embedded tissue blocks.

Inclusion criterion: All histopathologically proven cases of urothelial tumors in the urinary bladder. Exclusion criteria: Blocks could not be retrieved. Patients with history of chemotherapy and radiotherapy

Scoring of Ki-67 IHC [8]. The expression of Ki-67 was graded on the basis of the proportion of stained cells and their intensity: Score 0 / Negative: 0–10% cells. Score 1+ / Mild positive: 11–25% cells. Score 2+ / Moderate positive: 26–50% cells. Score 3+ / Strong positive: >50% cells.

Scoring of Her-2/neu IHC [9]. Interpretation of HER2/neu was done according to ASCO/CAP guidelines. Positivity was assessed as brown colour, cell membrane staining of malignant cells. Score 0 / Negative: No staining observed or <10%. Score 1+ / Negative: Faint staining or >10% cells. Score 2+ / Equivocal: Weak to moderate complete staining or >10% cells. Score 3+ / Strongly positive: Strong staining or >30% cells.

Statistical analysis: The collected data was entered into a Microsoft Excel spreadsheet. Continuous data was summarized as Mean ± SD, while percentage and proportion were calculated for qualitative data. Chi-square test and Fisher's exact test were used to find out the association between Ki-67 and Her2/neu IHC grading using SPSS 20 software. A p-value <0.05 was considered statistically significant.

Results

The patients included in the study were divided into age groups with a class interval of 12 years from 24 to 89 years. The mean age of the patients was 58.6 years. The largest number of cases, 19 (38%), was found in the 55–65-year age group, and 13 cases (26%) were found in the age group of 65–75 years. There were 7 cases (14%) in the 45–55-year age group and 6 cases (12%) in the 35–45-year age group.

The majority of the patients were male (82%), while only 9 patients (18%) were female. Of the total 50 cases, 33 (66%) had a history of smoking, and 17 cases (34%) were non-smokers. Among smokers, there was a male predominance, with 31 (75.6%) cases being male and 2 (22.2%) cases being female. The p-value for smoking habits was statistically significant (p = 0.002).

In the majority of cases, 48 (96%) TURBT were performed. Excision biopsy and radical cystectomy were each performed in 1 case (2%). The most common site of origin was the lateral wall, observed in 33 cases (66%), followed by the posterolateral wall in 13 cases (26%). The anterolateral and posterolateral walls were each the site of origin in 2 cases (4%).

The majority of cases, 25 (50%), were low-grade urothelial carcinoma (LGUC), followed by 22 (44%) cases of high-grade urothelial carcinoma (HGUC). There were two (4%) cases of squamous cell carcinoma (SCC) and one (2%) case of papillary urothelial neoplasm of low malignant potential (PUNLMP). Among HGUC cases, 12 (24%) showed muscle invasion, and 12 (24%) did not. Among LGUC cases, 5 (10%) showed muscle invasion, and 21 (42%) showed non-muscle invasion. PUNLMP is classified as LGUC non-muscle invasive. SCC is classified as HGUC muscle invasive.

IHC expression for Ki67 (nuclear staining) was graded as: Score 0 seen in 19 cases (38%), score +1 in 4 cases (8%), score +2 in 21 cases (42%), and score +3 in 6 cases (12%). Her-2/neu expression was seen as Positive (Score +3) in 13 (26%) cases, equivocal (Score +2) in 17 (34%) cases, and the remaining 20 (12% + 28%) cases were negative (Score +1 and Score 0) [Table 1].

In 12 high-grade muscle invasive tumors, Ki67 expression was: score 0 in one case (8.3%), score +1 in two cases (16.6%), score +2 in 5 cases (41.6%), and score +3 in 4 cases (33.3%). In low-grade muscle invasive tumors, out of 5 cases, Ki67 expression was score 0 in 3 cases and score +2 in 2 cases. In 12 high-grade non-muscle invasive tumors, Ki67 expression was: score 0 in 3 cases (25%), score +1 in 0 cases, score +2 in 8 cases (66.7%), and score +3 in 1 case (6.3%). In 21 low-grade non-muscle invasive tumors, Ki67 expression was: score 0 in 12 cases (57%), score +1 in 2 cases (9.5%), score +2 in 6 cases (28.5%), and score +3 in

1 case (5%). The correlation between Ki67 expression and muscle invasiveness among urothelial carcinoma cases was not found to be statistically significant, as the p-value was 0.11 [Table 2].

In high-grade muscle invasive carcinoma (12 cases), Her2/neu expression was: score +3 in 5 cases (41.6%), score +2 in 3 cases (25%), score +1 in 3 cases (25%), and score 0 in 1 case (8.3%). In low-grade muscle invasive tumors, out of 5 cases, Her2/neu expression was: score 0 in 2 cases, score +1 in 2 cases, and score +3 in 1 case. In 12 high-grade non-muscle invasive tumors, Her2/neu expression was: score +3 in 3 cases (25%), score +2 in 8 cases (66.6%), and score +1 in 1 case (8.3%). In 21 low-grade non-muscle invasive tumors, Her2/neu expression was: score 0 in 12 cases (57.1%), score +1 in 0 cases, score +2 in 5 cases (23.8%), and score +3 in 4 cases (19%). The correlation between Her2/neu expression and urothelial carcinoma cases was found to be statistically significant, as the p-value was 0.01 [Table 3].

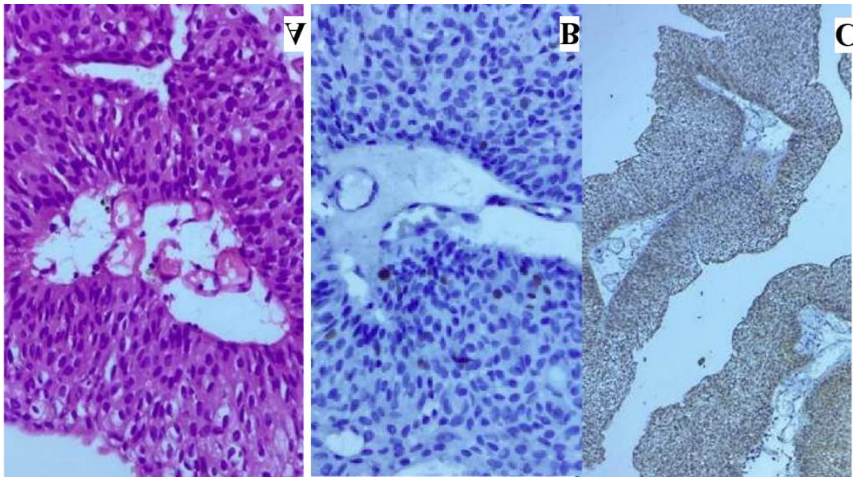

Figure 1: A: LGUC revealing cells with round to oval nuclei showing mild pleomorphism (H and E, 400X); B: LGUC showing Ki67 score 0 (10% staining) (IHC stain, 400X); C: LGUC showing Her2/neu overexpression (score 3+) (IHC stain, 100X).

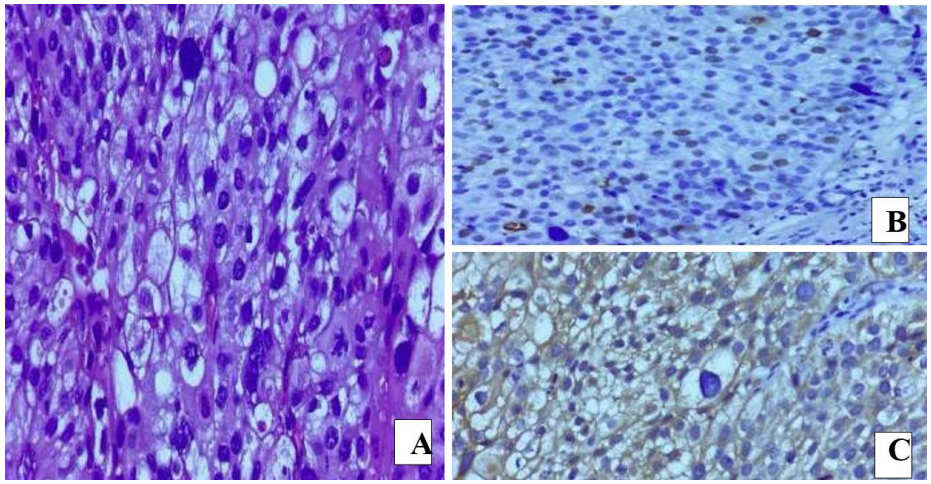

Figure 2: HGUC showing nuclear pleomorphism, nuclear enlargement with prominent nucleoli (H and E, 400X); B: HGUC showing Ki67 score 2+ (26-50%) (IHC stain, 400X; C: HGUC showing Her2/neu over expression (score 3+) (IHC stain, 400X).

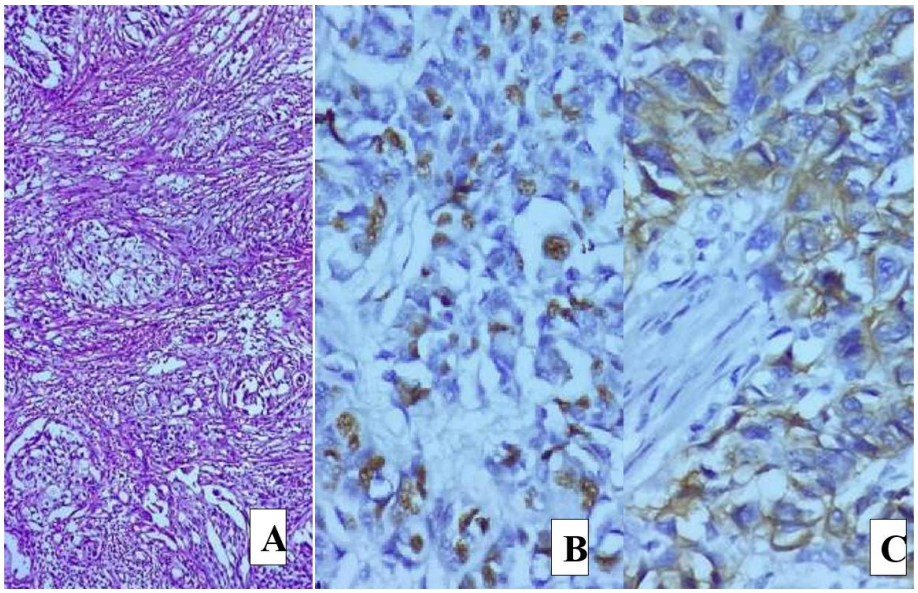

Figure 3: HGUC muscle invasive showing cells arranged in sheets showing nuclear pleomorphism with invasion of muscular layer (H and E, 400x); B: Ki67 score 3+ (>50% staining) (400x); C: Her2/neu overexpression (score 3+) (400x).

Discussion

Urinary bladder cancer is the most common malignancy involving the urinary system and the ninth most common malignancy worldwide. As per Indian Cancer Registry data, in men, it is the ninth most common cancer, accounting for 3.9% of all cancer cases in India. Men are affected more often than women (3–4:1) [1,2].

In our study, the maximum number of cases, i.e. nineteen cases (38%), were found in the age group of 55–65 years. The mean age of the patients was 58.6 years. Our study compares well with the studies conducted by Aslam M et al. [12] and Ibrahim et al. [8], having more cases in the age group of >60 years, i.e. 55.7% and 66.67%. Studies by Rehman et al. [10] and Haque S et al. [13] have a lower percentage of patients in the younger age group. This variation can be due to differences in the geographical area of study.

Cigarette smoking has long been recognized as a significant independent risk factor for bladder cancer. Of a total of 50 cases, 41 cases (82%) gave a history of smoking and 9 cases (18%) were non-smokers. These findings were in accordance with the findings of Haque S et al. [13] and Abid A et al. [10]; both found that the maximum number of patients (72%) in their studies had a habit of smoking.

In our study, the most common histological type of urothelial tumor diagnosed was low-grade tumor in 25 cases (50%). Twenty-two cases (44%) of high-grade tumor, two cases of squamous cell carcinoma (4%), and one case of PUNLMP (2%) were also reported. The most common histologic type in our study was low-grade urothelial tumor, which compares well with studies by Abid et al. [11] (24%) and Aslam M et al. [12] (64.56%). In contrast, high-grade urothelial tumor was reported as the predominant histological type in the studies by Ibrahim et al. [8] and Haque S et al. [13], i.e. 55% and 72%.

In our study, statistically significant results were seen in Ki67 expression: score 0 in 19 cases (38%), score 1+ in 4 cases (8%), score 2+ in 21 cases (42%), and score 3+ in 6 cases (12%) in relation to the grade of tumor (p-value 0.01). Ki67 expression in high-grade and low-grade tumors was 81.8% and 42.4%, respectively. These findings were in accordance with the findings of

Haque S et al. [13], where Ki67 expression in high-grade and low-grade UC was 80.6% and 50%, respectively. Most of the positive Ki67 expression cases were found in high-grade tumors.

Table 1: Demographic, risk factor, histopathological findings in cases (N=50)

Demographic and Histological findings	**No. of cases(n)**		**Percentage (%)**	
Total cases	50		100	
Age-				
<55 Years	16		32	
>55 Years	34		68	
Mean age:	58.6		-	
Median age:	55		-	
Range:	24-89		-	
Gender-				
Male	41		82	
Female	09		18	
M:F ratio				
Smoking	Smoker	Non-Smoker	Smoker	Non-Smoker
Males	31	10	75.6	24.4
Females	2	7	22.2	77.8
Specimen Type				
a. TURBT	48		96	
b. Excisional Biopsy	1		2	
c. Radical cystectomy	1		2	
Location				
a. Lateral wall	33		66	
b. Posterio-lateral wall	13		26	
c. Anterio-lateral	2		4	
d. Posterior wall	2		4	
Distribution of cases on the basis of histopathological diagnosis				
a. HGUC	22		44	
b. LGUC	25		50	
c. PUNLMP	1		2	
d. SCC	2		4	
Distribution of cases according to muscle invasiveness	**MI**	**NMI**	**MI**	**NMI**
HGUC	12	12	50	50
LGUC	5	21	19.2	80.7
Distribution of cases with respect to histological type (n)	**Ki67 Expression**		**Her-2/neu Expression**	
	0	**1+**	**2+**	**3+**	**0**	**1+**	**2+**	**3+**
[HGUC: high grade urothelial carcinoma, LGUC: low grade urothelial carcinoma, PUNLMP: Papillary urothelial neoplasm of low malignant potential, SCC: squamous cell carcinoma, MI: Muscle invasive, NMI: non muscle invasive, TURBT: Transurethral resection of bladder tumor]								

Table 2: Ki67 expression among the urothelial carcinoma cases according to muscle invasiveness(N=50)

TYPE			**Ki67**				**Total**
			0	**1+**	**2+**	**3+**	
Muscle invasive	**High**	**N**	1	2	5	4	**12**
		%	8.3	16.7	41.7	33.3	**100**
	Low	**N**	3	0	2	0	**5**
		%	60	0	40	0	**100**
Non muscle invasive	**High**	**N**	3	0	8	1	**12**
		%	25	0	66.7	8.3	**100**
	Low	**N**	12	2	6	1	**21**
		%	57	9.5	28.5	5	**100**
Total		**N**	**19**	**4**	**21**	**6**	**50**
		%	**38**	**8**	**42**	**12**	**100**

Table 3: Her2/neu expression among cases according to muscle invasiveness(N=50)

TYPE			Her2/neu				Total
			0	**1+**	**2+**	**3+**	
Muscle invasive	**High**	**N**	1	3	3	5	**12**
		%	8.3	25	25	41.7	**100**
	Low	**N**	2	2	0	1	**5**
		%	40	40	0	20	**100**
Non muscle invasive	**High**	**N**	0	1	8	3	**12**
		%	0	8	67	25	**100**
	Low	**N**	12	0	5	4	**21**
		%	57.1	0	23.8	19.1	**100**
Total		**N**	**15**	**6**	**16**	**13**	**48**
		%	**30**	**12**	**32**	**26**	**100**

Her2/neu expression showed statistically significant results: score 3+ in 13 cases (26.0%), equivocal (score 2+) in 17 cases (34.0%), and the remaining 20 cases (40.0%) were negative (score 1+ & score 0) in relation to tumor grade (p-value 0.01). Her2/neu expression in high-grade and low-grade tumors was 40.9% and 19%, respectively. These findings were in accordance with the findings of Jawad et al. [14] and Madhu et al. [2], both of whom found a significant association with Her2/neu overexpression in high-grade urothelial carcinoma as compared to low-grade carcinoma.

Conclusion

In conclusion, our study showed that cigarette smoking has long been recognized as a significant independent risk factor for bladder cancer. When compared to non-smokers, smokers have a fourfold greater risk of having bladder cancer [61]. Motivating patients and creating awareness regarding quitting the habit of smoking can help prevent cases of bladder cancer. This study demonstrated a statistically significant correlation between Ki67 and Her2/neu expression with tumor grade. Patients who exhibit a higher incidence of the disease may benefit from adjuvant Her2/neu treatment after undergoing radical cystectomy, provided that their Her2/neu status is evaluated.

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References

1. Biswas RR, Manga S, Guha D, Basu K, Karamakar D. An epidemiological study of cases of urothelial carcinoma of urinary bladder in a tertiary care center. J Krishna Inst Med Sci Univ. 2013;2(1):82-8.
2. Madhu K, Ponwang K, Suresh B, Atin S, Janak S, Sankhwar S, et al. Immunohistochemical expression of markers KI67 and HER. Int J Curr Res. 2016;8(12):43704-8.
3. Netto GJ, Amin MB. The lower urinary tract and male genital system. In: Kumar V, Abbas AK, Aster JC, Turner JR, Perkins JA, Robbins SL, et al., editors. Robbins and Cotran pathologic basis of disease. 10th ed. India: Reed Elsevier; 2021. p. 958-61.
4. Humphrey PA. The 2016 WHO classification of tumors of the urinary system and male genital organs—Part B: Prostate and bladder tumors. Eur Urol. 2016;70(1):106-19.

5. Hall MC, Chang SS, Dalbagni G, Pruthi RS, Seigne JD, Skinner EC, et al. Guideline for the management of nonmuscle invasive bladder cancer (stages Ta, T1, and Tis). J Urol. 2007;178(6):2314-30.
6. Babjuk M, Oosterlinck W, Sylvester R, Kaasinen E, Böhle A, Palou-Redorta J. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder. Eur Urol. 2008;54(2):303-14.
7. Raspollini LM, Taddei GL. Tumor markers in ovarian carcinoma. Int J Gynaecol Obstet. 2007;97(3):175-81.
8. Ibrahim BB, Mahmoud SA, Mohamed AA, Hanbuli HM. Expression of Her2/neu and Ki-67 in urothelial carcinoma and their relation to clinicopathological data: An Egyptian study. J Clin Diagn Res. 2021;15(3):6-9.
9. Iqbal BM, Buch A. Hormone receptor (ER, PR, Her2/neu) status and proliferation index marker (Ki67) in breast cancers: Their onco-pathological correlation, shortcomings and future trends. Med J DY Patil Univ. 2016;9(6):674-9.
10. Rahman P, Khan KH, Afroz S, Rahman MM, Rashid JS. Association of Ki-67 expression in radical cystectomy specimens of infiltrating urothelial carcinoma with histopathological parameters. Bangabandhu Sheikh Mujib Med Univ J. 2022;15(3):186-91.
11. Abid A, Sen S, Bandyopadhyay R. Clinicopathological study of urothelial neoplasms in urinary bladder with special reference to expression of Her2/neu and Ki67 in malignant lesion. Indian J Pathol Oncol. 2021;8(3):369-76.
12. Aslam M, Khadim MT, Hamdani SN, Ahsan F, Ali SS. Frequency of immunohistochemical expression of Ki67 in urothelial carcinoma bladder. Pak J Pathol. 2021;32(1):5-10.
13. Haque S, Dewan RK, Saleh S, Jennah SA, Jahan F, Akter F, et al. Histomorphological study of urinary bladder tumor and status of Her2/neu and Ki67 expression in urothelial carcinoma. J Histopathol Cytopathol. 2018;2(2):99-108.
14. Jawad NA, Ali AA, Kamal MS. Her2/neu and Ki67 immunohistochemical expression in transitional cell carcinoma of the urinary bladder. J Med Dent Sci. 2016;15(2):6-12.