Original Article

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Pathology Section

Significance of Ki67, BCL 2 Immunoexpression in Urothelial Carcinoma and their Association with Grading and Staging: A Descriptive Cross-sectional Study

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ABSTRACT

Introduction: Urinary bladder is the most common site of malignancy in the urinary tract and the most common type of cancer of urothelial origin is urothelial cell carcinoma. This subtype constitutes more than 90% of bladder cancer. Histological grade of tumour is one of the most important predictive parameter for prognosis and biological behavior of urothelial carcinoma. As some crucial discordances may arise between pathologists because of subjectivity, so immunohistochemistry can be used for accurate diagnosis and prediction of prognosis.

Aim: To study the immununohistochemical expression of B-Cell Lymphoma 2 (BCL 2) and Ki (Kiel)-67 markers and their association with tumour grade and stage in urothelial carcinomas.

Materials and Methods: A descriptive cross-sectional study was conducted in the Department of Pathology of a tertiary care R G Kar Medical College and Hospital, Kolkata, West Bengal, India. West Bengal, India, on a total of 75 cases from January 2018 to June 2019. The study population comprised of cases of urothelial lesions as diagnosed by cystoscopy, who underwent standardised operative procedures. Relevant sections were taken from different parts of the fixed specimens. Histopathological Examination (HPE) was done to confirm the presence or absence of non-invasive and invasive carcinoma, and the depth of invasive tumour cells for pathological staging. Evaluation of the level of cytological and architectural disorder at low and medium magnification (100X and 200X) were done for grading. Cytological disorder were defined as abnormalities in nuclear size, shape, and chromatin, while architectural disorder were defined as abnormalities in the orientation of the cells in relation to each other and to the basement membrane of the papillae. Immunohistochemical analysis was done for Ki67 and BCL 2 expression in paraffin embedded sections in all the cases. In this study, chi-square test was done with International Business Management(IBM) Statistical Package for Social Sciences (SPSS), as per which, the association between two variables is statistically significant if p value is <0.05.

Results: Out of total 75 cases (mean age: 67.3 years) analysed, 58 cases (77.3%) had Invasive type of bladder neoplasm, followed by low grade papillary urothelial carcinoma 10 (13.3%), and papillary urothelial neoplasm of low malignant potential 07 (9.3%). Ki67 expression in urothelial carcinoma has more significance because 59 cases (78.7%) showed high level expression (>20%) out of 75 cases, whereas only 12 (16%) cases show high level expression (4+) of BCL 2 marker.

Conclusion: Ki67 overexpression is seen more commonly, than BCL 2, in high grade tumours as well as in advanced stage. So Ki67 may be used as a marker to predict aggressive behavior and to differentiate low grade and high grade tumours also.

Keywords: B-cell lymphoma 2, Immunohistochemistry, Kiel-67 gene, Muscle invasion, Urinary bladder

INTRODUCTION

Bladder cancer was the 10th most commonly diagnosed malignancy worldwide, accounting for 549,393 new cases and 199,922 deaths in 2018 [1,2]. Urothelial cell carcinoma is the predominant histologic subtype of bladder cancer, contributing to more than 90% of bladder cancer cases [3]. On microscopic examination, the detection of the invasion of the tumour process to sub epithelial tissues is important to determine its effect on therapeutic measures as well as prognosis of the disease process [2,3].

The invasion of urothelial carcinoma to sub epithelial tissues proceeds in two stages: invasion of the lamina propria and invasion of the muscle layer. The detection of the former is difficult and that of the latter has great significance because of its influence on therapy and prognosis, both regarding recurrence as well as survival. 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 [1-5].

Stage of malignancy is an important determinant for a patient's outcome in most solid organ malignancies [4-6] Overall, the five-year survival drops to 38.5% for stage T2 as compared to 70% in stage T0 and T1 bladder cancer patients. However, histological grade is considered an equally, or even more, important prognostic factor in particular for the superficial urothelial neoplasms of the bladder, a category that includes tumours either with only lamina propria invasion or without invasion. It has been demonstrated that grade strongly correlates with recurrence and the presence of, or progression to muscle invasive disease (muscularispropria invasion) in superficial urothelial neoplasms [7].

This study discusses the current understanding of emerging biomarkers Ki 67 and BCL 2 and their potential clinical value in Bladder Cancer (BC) diagnosis, as prognostic indicators, and surveillance tools, as well as limitations to their incorporation into medical practices. Ki67 is a non-histone nuclear protein with a short life. It is strictly associated with cell proliferation, which is present during all active phases of the cell cycle (G1, S, G2, and mitosis). Ki67 is a preferably convenient biomarker for the proliferation status of tumour cells [8-10]. Recently, several studies continuously reported that Ki67 is an independent indicator to predict poor clinical outcomes of both no muscle invasive and muscle invasive BC patients. Apoptosis (programmed cell death) and the genes regulating this process (e.g., BCL 2) have recently become a focus of interest in the study of cancer development and progression.

The BCL 2 gene product plays a role as an inhibitor of apoptosis; it contributes to oncogenesis by suppressing signals that induce apoptotic cell death [11,12]. BCL 2 is a protein that helps control whether a cell lives or dies by blocking a type of cell death called apoptosis [13].

Therefore, the present study was conducted with the aim to compare and determine the association of Ki67 and BCL 2 immunoexpression in urothelial carcinoma as well as their significance in grading and staging.

MATERIALS AND METHODS

This descriptive cross-sectional study was conducted in the Department of Pathology, in collaboration with Department of Urosurgery, at R G Kar Medical College and Hospital, Kolkata, from January 2018 to June 2019 after approval from the Institutional Ethical Committee (RKC/Ethics/30).

Inclusion criteria: Those patients who presented clinically with symptoms suspicious of urothelial carcinomas like painless, gross haematuria, nocturia, dysuria or urgency of micturition and were radiologically diagnostic of urothelial neoplasms of urinary bladder. For histopathological confirmation, specimens of both Transurethral Resection of Bladder Tumour (TURBT) as well as radical cystectomy were examined.

Exclusion criteria: Those cases with mesenchymal tumours (angioma, myoma, fibroma, sarcoma etc.) arising from the bladder, primary adenocarcinomas arising from urachal remnant or areas of glandular metaplasia, pure squamous cell neoplasms, all inflammatory (inflammatory pseudopolyps, interstitial cystitis etc.) conditions in bladder, and all cases of bladder lithiasis were excluded from the study.

Total of 75 cases were included in the study following the inclusion and exclusion criteria.

Study Procedure

The medical history, radiological, and other investigation reports were checked. Cystectomy specimen or TURP chips of bladder was obtained from the Urosurgery Department in which site of tumour was also mentioned. All tissues were examined grossly and microscopically for appropriate pathological stage evaluation. The tumours were categorised as Ta (non-invasive urothelial carcinoma), Tis (urothelial carcinoma in situ), T1 (tumour involving lamina propria), T2 disease (tumour involving muscularispropria), T3 (Tumour invades perivesical soft tissue) or T4 (extravesical tumour directly invading any of the following: prostatic stroma, seminal vesicles, uterus,vagina, pelvic wall, abdominal wall).Nodal dissection specimens were received in separate packets and all lymph nodes examined microscopically, as both the number and the location of positive lymph nodes are important to asses prognosis [14].

Histopathological findings with grading is reported, grading of tumour was based on the evaluation of the level of cytological and architectural disorder microscopically [15]. Immunohistochemical (IHC) staining for Ki67 and BCL 2 were also done. Then association of Ki67 and BCL 2 with clinicopathological parameters were studied.

Poly-L-Lysine coated slides were prepared as follows: Poly-L-Lysine [Sigma chemicals] was dissolved in distilled water (i.e., 1:10 dilution) and then dried. Preparation of Tris (Trisaminomethane)- Ethylene Diamine Tetra-acetic Acid (EDTA) Buffer (Antigen-Retrieval buffer) was done.

Preparation of TRIS Buffer (Wash buffer) and % Hydrogen peroxide: 9.6 gm of TRIS (Molecular weight (Mol. wt.): 121.14) add 8.7 gms of Sodium chloride (NaCl) (MERCK, no: 7647-14-5) were dissolved in 1000 mL of distilled water. 97 mL of distilled water, 3 mL of H_2O_2 was added to make 3% of H_2O_2. Primary antibody was used according to the immunohistochemical markers. Preparation of 3,3- Diaminobenzidine (DAB) solution was done. Then following procedure is done for staining:

- Removal of Paraffin and Rehydration
- Antigen Retrieval-Unmasking of Antigen
- Inactivation of Endogenous Peroxidase with 3% hydrogen
- Primary Antibody Reaction in which the primary antibody was diluted to its optimal dilution in diluent. The diluent alone was used as a negative control. Than a positive control slide was also run.
- Secondary Antibody Reaction in which secondary antibody was diluted in the diluent to its optimal dilution.
- Counterstaining with Mayer's haematoxylin.

Ki67 scoring system and criteria: Cells stained for Ki67 were counted and expressed as a percentage. The percentage was determined by the number of Ki67 positive cells among the total number of counted tumour cells (at least 1000) with nuclear staining in each case. High expression of Ki67 was defined as ≥20% and low level expression was defined as less than 20% immunoexpression [16].

BCL 2 scoring system and criteria: Only cytoplasmic staining was scored as positive for BCL 2, regardless of the intensity of the stained cells. BCL 2 expression [17] was assessed as follows:

1. Negative (0)-No immunoreactivity detectable.
2. Weakly positive (1+) when less than 5% of the cells show positive for BCL 2.
3. Moderate positive (2+) 5 to 50% of tumour cells are positive.
4. Strong positive (3+) more than 50% of tumour cells are positive.

For positive controls of BCL 2 and Ki67 follicular lymphoma and normal tonsil were stained respectively.

STATISTICAL ANALYSIS

The statistical analyses were performed using the SPSS version 23.0 software program. Relationships among the clinicopathological factors and BCL 2, Ki67, expressions were analysed using chi-square test and Fisher exact t-test. The results were considered statistically significant if the p-value was ≤0.05.

RESULTS

The present study was done on total 75 cases who were admitted to the inpatient Department of Urosurgery, for bladder tumour and they underwent surgery. Mean age was 67.3 years (range was 42 to 86), [Table/Fig-1]. A total of 59 cases were above the age of 65 years and 16 cases were below this age. Gender distribution showed that most of the cases were male (72%) with a male to female ratio is 18:7. According to site of origin, most of the tumours were located in right and left lateral wall and posterior wall of bladder, together they comprises most of the common sites of origin of tumour [Table/Fig-2].

Age group (years)	Male, N (%)	Female, N (%)
<65	12 (16)	14 (18.67)
≥65	42 (56)	7 (9.33)
Total	54 (72)	21 (28)

[Table/Fig-1]: Show age and sex related incidence of cases; Total N=75 cases.

Site of tumour		Number (%)
Urothelial carcinoma (Total 75)	Anterior and right lateral wall	5 (6.67)
	Left lateral	18 (24)
	Left lateral and posterior wall	13 (17.3)
	Left lateral and Dome	5 (6.67)
	Right lateral	28 (37.3)
	Right and posterior wall	6 (8)
	Total	75 (100)

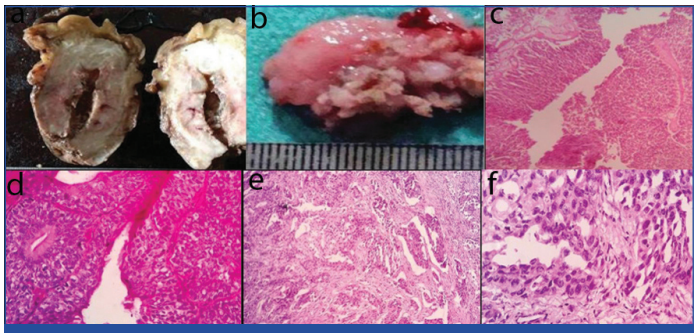
[Table/Fig-2]: Tumour site within the bladder; N=75 cases of Urothelial Carcinoma.

Distribution of different histologic variants were-invasive (77.3%), low grade and papillary urothelial neoplasm of low malignant potential. [Table/Fig-3] Gross examination [Table/Fig-4a,b] of specimens was

done. Low-grade lesions [Table/Fig-4c,d] have long, delicate papillae with minimal branching and fusing. At low magnification, they have a relatively orderly appearance, but at medium magnification mild pleomorphism and some loss of polarity is found. But in high grade carcinoma [Table/Fig-4e,f] the papillae may be fused, cellular disorder, nuclear size variation and irregular and pleomorphic nuclei are readily apparent at low to intermediate magnification.

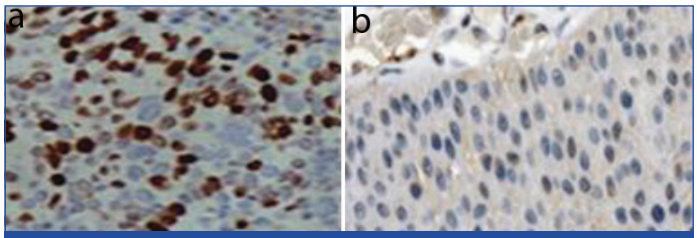
Histopathology	Frequency
Invasive urothelial carcinoma	58 (77.3%)
Low Grade Papillary Urothelial Carcinoma (LGPUC)	10 (13.3%)
Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP)	07 (9.3%)
Total	75 (100%)

[Table/Fig-3]: Distribution of different histologic variants.



[Table/Fig-4a-f]: (a) Gross image of specimen showing-bladder with thickened Wall (b) Transurethral Resection of Bladder Tumour (TURBT) Specimen (c) Photomicrograph of Low Grade Papillary Urothelial Carcinoma (H&E; 100X) showing long, slender papillae with minimal fusing or branching. Orderly architecture at low magnification (d) Low Grade Urothelial Carcinoma (H&E 400X) neoplastic urothelium lining fibrovascular cores (e) Photomicrograph of High Grade Urothelial Carcinoma (H&E 100X) showing complex, solid to fused papillae with architectural disorder. (f) Photomicrograph of High Grade Urothelial Carcinoma showing deep muscle invasion (H&E 400X).

Irregular prominent nucleoli and numerous mitoses, including irregular forms are typical. In [Table/Fig-5], high-level Ki67 [Table/Fig-5a] expression in nuclei of tumour cells and low level BCL 2 [Table/Fig-5b] cytoplasmic expression is shown. Total 24 cases of low grade carcinoma were studied and 15 cases showed high level Ki67 immunoexpression but only three cases showed high level immunoexpresion of BCL 2. There were 51 cases of high grade carcinoma, out of which 44 cases showed high level immunoexpression of Ki67 whereas only 9 cases showed high level immunoexpression of BCL 2.



[Table/Fig-5a-b]: (a) Microphotograph showing overexpression of Ki67 in Urothelial Carcinoma (IHC 400X),Nucleus of cells stained for Ki67 were counted (IHC 400X). (b) Microphotograph showing expression of BCL 2 in Urothelial Carcinoma (IHC 400X) cytoplasmic staining was scored as positive for BCL 2, regardless of the intensity of the stained cells.

Association of Ki67 expression with clinicopathological parameters were summarised in [Table/Fig-6]. The Ki67 expression was more in advance cases. Association between stage of tumour with both Ki67 and BCL 2 expression was also studied. The p-value is greater than 0.05 (0.6430) in BCL 2 expression, so there is no association between BCL 2 expressions and staging of urothelial carcinoma cases. Association between histological grade of urothelial tumour and BCL 2 expression was also evaluated. It was concluded that since the p value is greater than 0.05, there is no association between BCL 2 expressions and grading of urothelial carcinoma cases. Whereas association between histopathological grade of bladder tumour and Ki67 expression was also studied. Analysis of data suggested an association between Ki67 expressions with grading (p-value 0.019) of urothelial carcinoma cases. High grade carcinoma cases showed very high expression.

Variables	Ki67 expression		p-value	BCL 2 expression				p-value
	Low level	High level		–	+	++	+++	
Patients	16	59	–	27	20	16	12	–
Gender								
Male	12	42	0.7631	20	14	11	09	0.9718
Female	04	17		07	06	05	03	
Age								
≥65	07	42	**0.0408**	17	13	11	08	0.9837
<65	09	17		10	07	05		
Tumour side								
Right	07	32	0.4564	16	7	09	03	0.1289
Left	09	27		11	13	07	09	
Addiction								
Smoker	12	41	0.6677	17	15	10	11	0.2591
Non smoker	04	18		10	5	6	1	
Multiplicity								
Solitary	12	49	0.4635	24	18	10	09	0.1087
Multiple	04	10		3	2	6	03	
Tumour invasion								
Non invasive	06	11	0.1100	03	05	04	4	0.3917
Invasive	10	48		24	15	12	8	
Histological grade								
High	07	44	**0.0190**	16	15	11	9	0.6430
Low	09	15		11	5	5	3	
Tumour size								
>4 cm	06	24	0.8179	9 12		6	3	0.1717
≤4 cm	10	35		18	8	10	9	
Nodal status								
PNo-NX	15	55	0.6045	25	19	15	11	0.9821
PN+	01	04		2	1	1	1	

[Table/Fig-6]: Association of Ki67 and BCL 2 expression with clinicopathological parameters.

DISCUSSION

In this study, association of clinicopathological features with molecular marker Ki67 and BCL 2 expression was evaluated systematically in different type of bladder cancer. It has been found that low level Ki67 is commonly seen in low grade bladder carcinoma and high Ki67 expression was associated with the more aggressive clinical stage and larger size tumour in bladder cancer patients. Ki67 expression was not strongly associated with age, gender, and tumour site in these patients. Some researchers investigated the relationships between the Ki67 and distant metastases [18]. In this study of 75 cases, it has been found that Ki67 expression was elevated with advanced age and with the progression of tumour grade (p-value=0.0190). The largest series to date, reported by Jeon HG et al., [19] on 107 Upper urinary Tract Urothelial Carcinoma (UTUC) patients, revealed that Ki67 overexpression was an independent risk factor.

A number of studies have examined the role of BCL 2 as a prognostic factor of bladder cancer outcome. Most studies did not identify a prognostic significance of this marker [19,20]. Nakopoulou L et al., found that a loss of BCL 2 positivity had an unfavorable prognosis; however, in multivariate analysis, there was no independent prognostic value [20]. In contrast, Lipponen PK et al., [21] reported that a heterogeneous group of tumours with BCL 2-positive non-basal cells had an unfavorable prognosis but in multivariate analysis,

expression of BCL 2 had no independent prognostic value. In a study by Kong G et al., all find the BCL 2 positive rate was much lower than that reported previously (7% to 69%) and was not related to tumour grade or pathological stage [22].

Nakopoulou L et al., found a strong positive correlation of mitotic indices and Ki67 with tumour grade [20], Ki67 ≥59% and mitotic count ≥36.50 per 10 High Power Field (HPF), if proven seem to be promising and reliable indicators to assess muscle invasion in superficial bladder biopsies and TURBT samples where muscle is not resected. Epstein JI et al.found recurrence and tumour progression the expression of Ki67 was significantly higher in high grade tumours [14]. Tony T et al., opined that combined use of p53 and Ki67 immunomarkers in urinary bladder carcinomas may provide additional prognostic information along with histological grading and staging [23].

Ogata DC et al., found high grade Transitional Cell Cancer (TCC) had a higher frequency of positivity for this antibody [24]. Matsumoto H et al., found comparing pathological stage and histological grade, histological grade correlated with increasing stage and Ki67 expressions in bladder UCs increased with pathological stage and histological grade and that it was possible to obtain more accurate information about the biological behavior of UC by evaluating these parameters together with morphological findings [25].

Stanton MJ et al., and Wang L et al., also found Ki67 labelling index as an independent predictor of tumour, recurrence for patients with primary superficial low grade bladder cancers. They concluded that determination of tumour growth kinetics by Ki67 index antibody might help to identify more biologically aggressive tumours and help physicians select patients with superficial bladder cancer who require more intense follow-up and/or prophylactic intravesical chemotherapy instillation [26,27].

Limitation(s)

The study sample was limited. This study needs to be extrapolated to a large population-based sample with financial support sufficient enough to find relationship of expression of biomarker with grade and stage of tumour and findings need to be confirmed by a larger prospective cohort study.

CONCLUSION(S)

In conclusion, tumour stage and grade is not associated with BCL 2 but there was a strong similarity with Ki67 proliferation index. This study confirmed the significant associations between Ki67 expression with some clinicopathological features (advanced age) and histological grade in bladder carcinoma, so Ki67 immunoexpression may be helpful to identify patients at high risk who may benefit by adjuvant therapies.

REFERENCES

[1] Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder cancer incidence and mortality: A global overview and recent trends. Eur Urol 2017;71(1):96-08.
[2] Bancroft J D, Gamble M. Theory and Practice of Histological Techniques. 6th Edition. 20 08 Churchill Livingston e. pg. 319.
[3] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012;62(1):10-29.
[4] Lin Z, Kim H, Park H, Kim Y, Cheon J, Kim I. The expression of bcl-2 and bcl-6 protein in normal and malignant transitional epithelium. Urol Res. 2003;31(4):272-75.
[5] Ro JY, Ayala AG, el-Naggar A. Muscularis mucosa of urinary bladder. Importance for staging and treatment. Am J Surg Pathol. 1987;11(9):668-73.
[6] Philip AT, Amin MB, Tamboli P, Lee TJ, Hill CE, Ro JY. Intravesical adipose tissue: A quantitative study of its presence and location with implications for therapy and prognosis. Am J Surg Pathol. 2000;24(9):1286-90.
[7] Bochner BH, Hansel DE, Efstathiou JA, Konety B, Lee CT, McKiernan JM, et al. AJCC Cancer Staging Manual. 8th edition. Switzerland: Springer; 2017. p. 758.
[8] Koss LG, Melamed MR. Koss' Diagnostic Cytology and its histopathologic bases. 5th edition. New York: Lippincott Williams and Wilkins; 2006. P742.
[9] Munoz JJ, Ellison LM. Upper tract urothelial neoplasms: Incidence and survival during the last 2 decades. J Urol. 2000;164(5):1523-25.
[10] Ploeg M, Aben KK, Kiemeney LA. The present and future burden of urinary bladder cancer in the world. World J Urol. 2009;27(3):289-93.
[11] Rouprêt M, Zigeuner R, Palou J, Boehle A, Kaasinen E, Sylvester R, et al. European guidelines for the diagnosis and management of upper urinary tract urothelial cell carcinomas: 2011 update. EurUrol. 2011;59(4):584-94.
[12] Feig BW, Ching CD. The MD Anderson Surgical Oncology handbook. 5th edition pg. 614-621.
[13] Witjes JA, Bruins HM, Cathomas R, Compérat EM, Cowan NC, Gakis G, et al. European association of urology guidelines on muscle-invasive and metastatic bladder cancer: Summary of the 2020 guidelines. Eur Urol. 2021;79(1):82-04.
[14] Epstein JI, Amin MB, Reuter VE. Bladder Biopsy Interpretation. Philadelphia: Lippincott Williams & Wilkins; 2004. p.79.
[15] Kirkali Z, Chan T, Manoharan M, Algaba F, Busch C, Cheng L, et al. Bladder cancer: Epidemiology, staging and grading, and diagnosis. Urology. 2005;66(6 Suppl 1):4-34. Doi: 10.1016/j.urology.2005.07.062.
[16] Krabbe LM, Bagrodia A, Lotan Y, Gayed BA, Darwish OM, Youssef RF, et al. Prospective analysis of Ki-67 as anindependent predictor of oncologic outcomes in patients with high grade upper tract urothelial carcinoma. J Urol. 2014;191(1):28-34.
[17] Saegusa M, Takano Y, Kamata Y, Okayasu I. Bcl-2 expression and allelic loss of the p53 gene in gastric carcinomas. J Cancer Res Clin Oncol. 1996;122(7):427-32.
[18] Lan YJ, Chen H, Chen JQ, Lei QH, Zheng M, Shao ZR. Immunolocalization of vimentin, keratin 17, Ki67, involucrin, beta-catenin and E-cadherin in cutaneous squamous cell carcinoma. Pathol Oncol Res. 2014;20(2):263-66. Doi: 10.1007/s12253-013-9690-5 PMID: 23999979.
[19] Jeon HG, Jeong IG, Bae J, Lee JW, Won JK, Paik JH, et al. Expression of Ki-67 and COX-2 in patients with upper urinary tract urothelial carcinoma. Urology. 2010;76(2):513-17.
[20] Nakopoulou, L, Vourlakou C, Zervas A. The prevalence of bcl-2, p53, and Ki-67 immunoreactivity in transitional cell bladder carcinomas and their clinicopathologic correlates. Hum Pathol. 1998;29(2):146-54.
[21] Lipponen PK, Aaltomaa S, Eskelinen M. Expression of the apoptosis suppressing bcl-2 protein in transitional cell bladder tumours. Histopathology. 1996;28(2):135-40.
[22] Kong G, Shin KY, Oh YH, Lee JJ, Park HY, Woo YN, et al. Bcl-2 and p53 expressions in invasive bladder cancers. Acta Oncol. 1998;37(7-8):715-20.
[23] Wu TT, Chen JH, Lee YH, Huang JK. The role of bcl-2, p53, and ki-67 index in predicting tumor recurrence for low grade superficial transitional cell bladder carcinoma. J Urol. 2000;163(3):758-60.
[24] Ogata DC, Marcondes CAPR, Tuon FF, Busato WFS Jr, Cavalli G, Czeczko LEA. Superficial papillary urothelial neoplasms of the bladder (PTA E PT1): Correlation of expression of P53, KI-67 and CK20 with histologic grade, recurrence and tumor progression. Rev Col Bras Cir. 2012;39(5):394-400.
[25] Matsumoto H, Wada T, Fukunaga K, Yoshihiro S, Matsuyama H, Naito K. Bax to Bcl-2 ratio and Ki-67 index are useful predictors of neoadjuvant chemoradiation therapy in bladder cancer. Jpn J Clin Oncol. 2004;34(3):124-30.
[26] Stanton MJ, Maxted W. Malacoplakia: A study of the literature and current concepts of pathogenesis, diagnosis and treatment. J Urol. 1981;125(2):139-46.
[27] Wang L, Feng C, Ding G, Zhou Z, Jiang H, Wu Z. Relationship of TP53 and Ki67 expression in bladder cancer under WHO 2004 classification. J BUON 2013;18(2):420-24.

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