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# Urothelial neoplasms: a review of pathological reporting practices

**Prashant Basavaraj Mahalingashetti[1,*], Radha Madhavi T[2], Balachandra Bhat[3], Swaminathan Boobalan[4]**

[1,3,4]Assistant Professor, [2]Resident, Dept. of Pathology, PESIMSR, Andhra Pradesh

***Corresponding Author:**
Email: pmshetti49@gmail.com

## Abstract

**Introduction:** Urothelial carcinoma has observed great strides of advances in the field of diagnostics and therapeutics. On the other hand, the last decade witnessed an upheaval in pathological grading which seems to settle down in the recent times.

**Aims and Objectives:** Our study intends to evaluate pathology practices in reporting papillary urothelial carcinomas and highlight the features which can define prognosis and guide molecular studies.

**Materials and Methods:** A consecutive of 32 cases of papillary urothelial carcinoma reported over duration of 2 years were collected. Histopathological slides were reviewed applying WHO/ISUP 2004 diagnostic criteria and compared with primary diagnosis.

**Results:** High grade papillary urothelial carcinoma formed the largest group with 14 cases. Concordance between original diagnosis and review diagnosis was seen in 19 cases only. Invasion into underlying tissue was missed in 6 cases. Associated features like carcinoma in situ, lymphovascular invasion, perineural invasion and stalk invasion were missed in primary reports.

**Conclusion:** As grade and stage dictate prognosis and management plans, it is essential to release veritable reports. The findings emphasize the need to generate a consensus report with multiple opinions.

**Keywords:** Urothelial neoplasms, Invasion, Tumor Grade

## Introduction

The classification and grading of urothelial neoplasms had witnessed imbroglio over many decades. Disparity in nomenclature and grading of bladder tumors can result in difficulties with clinical management and accurate collection of cancer statistics.[1,2,3,4] The morphological criteria useful for grading has been continuously refined and updated.[4] The earlier 1973 WHO grading system suffered from a poor inter observer reproducibility and lumping of tumors in the intermediate category.[4,5] In 2004, the WHO adopted the 1998 ISUP consensus classification system, and the 2004 WHO/ISUP system emerged.[2,3,5,6,7] This system separates papillary urothelial neoplasms into 4 categories: Papilloma, Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP), Low Grade Papillary Urothelial Carcinoma (LGPUCa), High Grade Papillary Urothelial Carcinoma (HGPUCa).[3,5,7,8] Revered bodies like American Urological Association and College of American Pathologists have set management guidelines utilizing WHO 2004 system.[3] Despite its shortcomings, European Association Urology (EAU) utilizes WHO 1973 grading system claiming it to be clinically confirmed, robust, widely used and reasonably reproducible.[4,5,8,9] Some recommend use of both 1973 WHO and 2004 WHO classification.[8]

## Objective

To analyze diagnostic accuracy and reproducibility in reporting urothelial neoplasms following implementation of WHO/ISUP 2004 consensus classification. This study addresses the trend in pathology practices of reporting urothelial neoplasms at a single, tertiary care, academic facility. We also attempt to describe other pathologic variables noticed on histological sections.

## Materials and Study

The histopathology archives were searched for all cases with the diagnosis of urothelial carcinoma rendered between Jan 2013 and August 2015. Other bladder malignancies such as squamous cell carcinoma, adenocarcinoma, neuroendocrine carcinoma, metastatic prostatic carcinoma and undifferentiated carcinoma were excluded from the study group. Recurrent carcinomas were not included in this study. Clinical and pathological data from department records, and Hematoxylin and eosin stained sections were retrieved for all cases. A week long literature review and erudite discussion was scheduled before review of slides. All cases were reviewed for consensus histological impression without the knowledge of previous diagnosis, employing currently recommended WHO/ISUP 2004 grading system Table 1.[10,11] Other histological variables such as volume of tissue sent in each case and number of blocks prepared was noted. Mitotic rate in areas with highest density, presence of lamina propria and muscularis propria were also assessed on the sections.

**Table 1: Histologic features of Papillary urothelial lesions[10,11]**

| Feature | Papilloma | PUNLMP | LGPUCa | HGPUCa |
|---|---|---|---|---|
| Papillae | Delicate | Delicate | Fused | Fused |
| Polarity of cells | Normal | Normal | Minimal crowding, minimal loss | Frequent loss |
| Nuclear size | Normal | Uniformly enlarged | Enlarged with slight variation | Enlarged with moderate-marked variation |
| Nuclear shape | Normal | Elongated | Round-oval | Round-oval |
| Nuclear chromatin | Fin | Fin | Mild variation | Moderate-marked variation |
| Nucleoli | Absent | Absent | Inconspicuous | Prominent |
| Mitoses | Absent | Rare, basal | Occasional, at any level | Frequent, at any level |
| Umbrella cells | Present | Present | Usually present | May be present |

## Results

A total of 32 cases were identified. All the cases were diagnosed on TUR bladder biopsy. The mean age of patients was 63.5 years (range 22-90 years). Males formed the larger group with 26 cases. Eight of these were diagnosed as Infiltrating Urothelial carcinoma with no papillary structures (Fig. 1d). The comparison between primary diagnosis and review diagnosis is demonstrated in Table 2. The original diagnosis of HGPUCa was confirmed in all the 14 cases. Slide review revealed missed lamina propria invasion in 3 cases and both muscularis and lamina invasion in two. The review illustrated associated findings like carcinoma in situ (CIS) in 3 cases, perineural invasion in 2 cases and lymphovascular invasion in 5 cases which were missed in original reports (Fig. 3). CIS, perineural invasion, lymphovascular invasion and squamous metaplasia(4 cases) were seen associated with HGPUCa only. PUNLMP was seen alongside in 2 cases of HGPUCa and a case of LGPUCa (Fig. 1a). Areas of LGPUCa were noted in two cases of HGPUCa. All the 3 LGPUCa had a low mitotic rate of 1/hpf, while in others it varied from 2-10/hpf. In five cases mitotic rate could not be ascertained with confidence due to fulcurisation artefact.

**Table 2: Comparison of Review diagnosis with Primary diagnosis of urothelial neoplasms**

| | | Review Diagnosis | | | | | |
|---|---|---|---|---|---|---|---|
| | | LGPUCa | | HGPUCa | | | Infiltrating UCa |
| Primary Diagnosis | | Non Invasive | Lamina Invasion | Non Invasive | Lamina Invasion | Both Invasion | |
| PUNLMP | | 1 | 0 | 0 | 1 | 0 | 0 |
| LGPUCa | Non Invasive | 2 | 1 | 1 | 0 | 1 | 0 |
| | Muscularis Invasion | 1 | 0 | 0 | 0 | 2 | 0 |
| HGPUCa | Non Invasive | 0 | 0 | 3 | 2 | 2 | 0 |
| | Lamina invasion | 0 | 0 | 0 | 4 | 0 | 0 |
| | Muscularis Invasion | 0 | 0 | 0 | 0 | 1 | 0 |
| | Both invasion | 0 | 0 | 0 | 0 | 2 | 0 |
| Infiltrating UCa | | 0 | 0 | 0 | 1 | 1 | 6 |
| Total = 32 | | 4 | 1 | 4 | 8 | 9 | 6 |

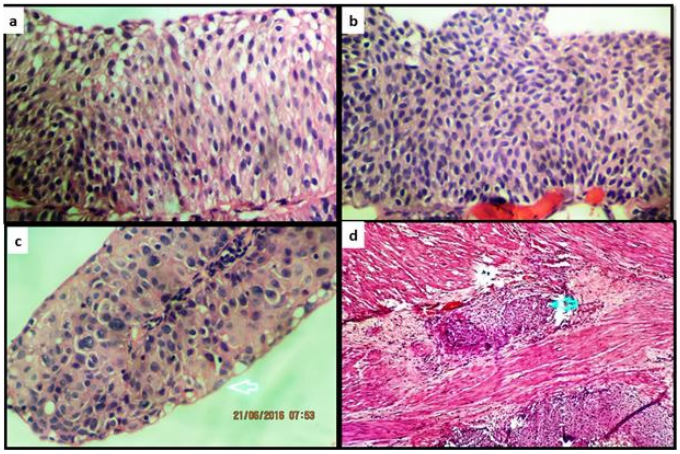


**Figure 1: a, Papillary urothelial neoplasm of low malignant potential (hematoxylin-eosin, original magnification X400) b, Low grade papillary urothelial carcinoma (hematoxylin-eosin, original magnification X400) c, High grade papillary urothelial carcinoma (hematoxylin-eosin, original magnification X400) d, Infiltrating urothelial carcinoma (hematoxylin-eosin, original magnification X100)**

## Discussion

Bladder cancer is the most common malignancy of the urinary tract. The worldwide age standardized rate is 10.1 per 100000 for men and 2.5 per 100000 for women. The global mortality rate is 4 per 100000 among men and 1.1 per 100000 among women.[9] Papillary urothelial neoplasms constitute about half of all bladder tumors.[1,5] TNM classification describes tumors confined to mucosa as Ta, tumors with submucosal invasion as T1 and T2 by invasion of muscularis propria.[5,7,12,13]

Nearly 50% of the cases in the present study had discrepancy on review diagnosis for histology type (papillary vs non-papillary), grade or invasion. Coblentz et al found that 18% of specimens with diagnosis of urothelial carcinoma had significant discrepancies in diagnoses, stage, grade or histologic tumor type when a second pathologist rendered an opinion.[5] Errors due to fulcurisation effect, tangential cutting, inadequate exposure of tissue bits, improper orientation of TURB bits can also cause difficulties in interpretation of slides.[1] Inter observer variability exist particularly in classifying dysplasia versus CIS, stage T1 versus Ta tumors and grading of the tumors despite well-defined criteria. Consequently, EAU guidelines recommend review of slide, particularly for T1, CIS and high grade tumors.[9]

Four cases that had been originally diagnosed as LGPUCa were reclassified as HGPUCa. Urothelial neoplasms frequently demonstrate areas of heterogeneity, making assignment of grade problematic.[2,5,8] Hence, it is plausible that a small area of high grade was ignored leading to misinterpretation.[2] Many authors suggest that grading of papillary urothelial neoplasms should be based on the worst grade present. On the other hand, Chang et al and others have suggested a scoring system combining scores for primary and secondary patterns akin to Gleason scoring system of prostatic carcinoma.[1] As aggressive management is advocated in recent times for HGPUCa, accurate grading remains at the fore of histological evaluation.[2]

Despite provision of detailed histologic criteria for the categories in the WHO 2004 system, improvement in intraobserver and inter observer variability has not been documented.[5,8] MacLennan et al curtly suggest to re-establish 1973 WHO as the international standard for grading urothelial papillary neoplasms.[5] Cheng et al proposed a new four tier grading system and claim to have incorporated strengths of both 1973 and 2004 WHO grading systems.[8]

Nearly a half of papillary urothelial tumors of any grade with associated CIS progress to muscle invasive disease. It also places patient at increased risk for death.[12,13] The current classification has expanded the category of carcinoma in situ to include those with single atypical cells growing in a pagetoid manner. Thus full thickness involvement of urothelium by atypical cells is not a prerequisite for its diagnosis.[3,4] Inter observer variability is high with CIS. Such a lesion is amenable to be missed.[3,4] None of LGPUCa showed coexistence of CIS in the present study. The likelihood of detecting CIS in low risk tumors is extremely low (<2%).[9]

On routine stain, capillary density of lamina on routine stain varying from 5 to 45 vessels per high power field was noted. However, the capillaries density could be estimated only in twelve cases. Crush artifact, fragmentation of tissue and absence of adequate lamina in few hindered the counting of vessels in rest. Curiously, the three cases with highest capillary density were those associated with CIS. Previous review has highlighted the increased frequency of tissue edema, vascular ectasia and proliferation of small capillaries in lamina of CIS.[4] Tumor angiogenesis involves over expression of angiogenic factors. Angiogenesis is

quantified by micro vessel density which is immunohistochemically stained.[14]

Previous studies point to high upstaging rate of 23% in high grade Ta tumors and 30% in T1 tumors respectively on repeat TUR. These numbers argue for repeat TUR resection 2-6 weeks after the initial resection for all high grade Ta or T1 tumors.[9,12,13] Our observation of invasion in 81% of HGPUCa too infers that it has a significant frequency of invasion, recurrence and progression to muscle invasive malignancy.[3,13] We opine that HGPUCa must be evaluated with high index of suspicion for invasion.

T1 tumors mandate second TUR as it is compounded that 30% of these will be upstaged in second TUR.[13] This underlies underscoring muscular invasion in reports. Sub staging of T1tumors has been proposed by few authors based on extent of invasion into lamina or using muscularis mucosae as landmark.[12,13,15] However, it was not attempted in our study as muscularis mucosa was not identified in many biopsy specimens. Sub staging T1 tumors is yet to be validated and is currently not advocated.[12]

Diagnostic difficulties associated with recognition of lamina propria invasion are due to tangential sections, poor tissue orientation, obscuring inflammation, thermal injury, and pseudoinvasive nests of benign proliferative urothelial cells.[12] When the biopsy contains no underlying stroma, or the stromal tissue is cauterized to annotate invasion, WHO recommends to report it as pTx.[15] The tumor cells invade as single cells or form irregular clusters (Fig. 2b and 2c). Retraction artifact around invasive clusters is a useful feature for diagnosing stromal invasion (Fig. 2b). Curiously, invading cells acquire abundant eosinophilic cytoplasm aptly termed "paradoxical differentiation" (Fig. 2a).[12] The smooth contour of preserved basement membrane is seen beneath noninvasive nests. A parallel array of thin walled vessels often line the basement membrane in noninvasive neoplasms, while these vessels are absent next to invasive nests.[12] We observed thin capillaries insinuating into overlying epithelia in three of high grade papillary urothelial carcinoma cases(Fig. 2d). Such phenomenon has been observed by previous authors and has been termed as "vascular entrapment".[15]

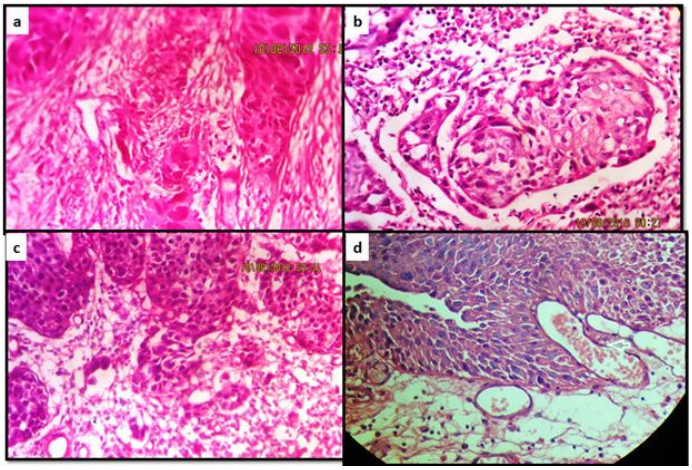


**Fig. 2: a, Paradoxical differentiation of infiltrating cells having abundant cytoplasm (hematoxylin-eosin, original magnification X400) b, Retraction artifact around invading nests in lamina (hematoxylin-eosin, original magnification X400) c, Invasion in the form of single cell (hematoxylin-eosin, original magnification X400) d, Vascular entrapment by papillary urothelial epithelia ( hematoxylin-eosin, original magnification X400)**

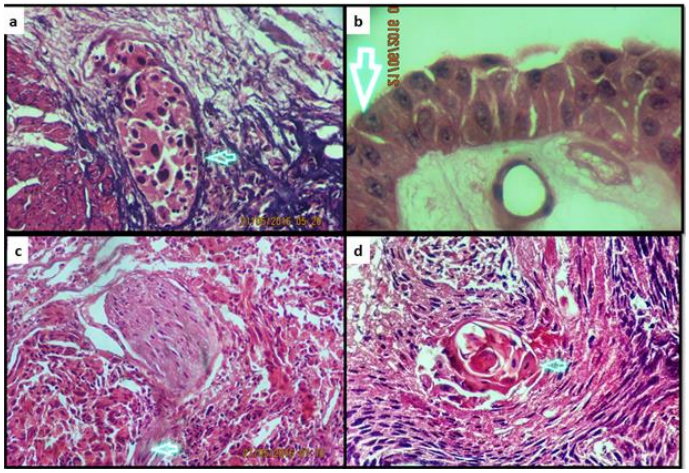


**Fig. 3: a, Lymphovascular invasion(hematoxylin-eosin, original magnification X400) b, Carcinoma in situ (hematoxylin-eosin, original magnification X400) c, Perineural invasion (hematoxylin-eosin, original magnification X400) d, Squamous metaplasis (hematoxylin-eosin, original magnification X400)**

Stromal response to invading tumor may be myxoid, fibrous, desmoplastic or inflammatory. Often this response is not uniform.[12,15] The lamina propria has the tendency to display response against large scale invasion only. Desmplastic host response is completely absent in cases with CIS. The pathologist fails to recognize microinvasive carcinoma in such cases as a band like infiltrate often found blurs the epithelial interface.[15] Occasionally stage pT1 tumors present as invasion into the fibrovascular core of papillary structures.[15] Under staging of T1 tumors has significant implications on patient management.[12]

The often quoted study by van der Mijden et al of 1400 patients highlights that 10% of patients originally staged T1 were found to have evidence of muscle invasion simply on pathology slide review.[13] Other reviews quote a number ranging from 0% to 17% for upstaging to muscle invasive tumor.[15]

The presence of muscle is an important indicator of adequately performed resection. Muscularis was not identified in 16 cases of the present study. As expected, this hinders accurate staging of tumors. Repeat TUR is suggested in tumors that lack detrusor muscle to assure accurate staging. Also the risk of residual tumor and muscle invasive disease when the resection is incomplete is significant.[9,13] Distinction between pT1 and pT2 urothelial carcinoma is rendered difficult in cases with hyperplasia of muscularis mucosae, replacement of detrusor muscle by desmoplastic reaction and carcinoma localized to bladder neck where detrusor muscle is very superficial. In the latter scenario, pT2 tumor can be mistaken for invasion of muscularis mucosae.[15]

EAU guidelines of 2011 recommend that complete and correct TUR is essential for the prognosis of the patient. The specimen should contain a part of the underlying bladder wall. Pathology request should contain cystoscopic features of the tumor (size, site, number and appearance) and mucosal abnormalities. A bladder diagram is recommended. Cauterization must be avoided as much as possible to prevent tissue destruction.[9,12] In the present study deep muscle biopsy was sent in separate container in 16 cases only. As practiced in these cases, tumor and deep tumor base biopsy should be submitted in separate containers to facilitate the detection of deep muscle invasion.[12]

The recurrence and progression of urothelial tumors is influenced by tumor multifocality, histological grade, tumor size, recurrence status, coexistence of carcinoma in situ, presence or absence of lymphovascular invasion, presence and stage of tumors.[5,7,12,13]

The dimensions of tumor were provided for only seven of our cases along with request forms. Many reviews have reported the influence of tumor size on stage. It has also been shown that 35% of superficial tumors larger than 5 cm progress to T2, compared to 9% of smaller superficial tumors.[13] Even though tumor size can be roughly estimated based on the volume of TUR specimen sent, radiology can assess the size better.[15]

Presence of lymphovascular invasion confers an increased risk of death as high as 70%. Identification of lymphovascular invasion can be difficult because of interobserver variability as it is confused with retraction artifact.[13] Most studies suggest that lymphovascular invasion confers inferior survival in patients with urothelial carcinoma. Five year survival for tumor with lymphovascular invasion is 65%, compared to 87% for that without.[12]

High throughput molecular techniques have unraveled dual pathogentic pathway for urothelial carcinogenesis. Most common genetic alterations in papillary neoplasms are loss of heterozygosity on 9q with activating mutations in HRAS and FGFR3. HGPUCa have in addition p53 mutations.[4,13,14] The concomitant presence of low grade neoplasms and precursor lesions like papilloma as exemplified in 5 of

our cases reflects a multistage tumorigenesis. On the other hand, CIS with early acquisition of p53 mutation progresses to muscle invasive non-papillary carcinomas.[4,14]

European Organisation for Research and Treatment of Cancer has developed a risk model with a scoring system incorporating six significant factors: number of tumors, tumor size, T category, tumor grade, prior recurrence rate and presence of concomitant CIS. The total scores are stratified into four categories reflecting the probabilities of recurrence and progression at 1 and 5 years.[9,13] These easily accessible histological variables help urologist to apply these tables on routine cases.

The best grading system should be easy to apply, reproducible and divide tumors into groups with different biological characteristics.[16] It allows for valid comparison of treatment results among various centres.[8] Our results are inimical to the 2004 WHOS/ISUP guidelines proposed for classification of urothelial carcinoma. An ideal grading system still eludes pathologists due to long standing lack of agreement. Consistent and standardized pathological evaluation is essential for comparison of treatment trials. This calls to pursue better and new parameters to improve risk stratification. Molecular markers have no significant clinical application at present. Other parameters such as blood group antigens, tumor associated antigens, proliferation, oncogenes, tumor angiogenesis have been investigated.[13]

Analysis of mitotic rate in our study highlighted the inherent difficulties presented due to fulcurisation artifact, fixation artifact, tangential cutting. The singed tissue fragments could be identified in all cases. It is advised not to use cautery while cutting and intended use of IHC for proliferation markers. Biomarkers such as PCNA(Proliferating Cell Nuclear Antigen) and Ki67 represent loss of cell cycle control and link with progression, aggression and prognosis of urothelial carcinomas.[6,14] Cina et al examined staining patterns of immuno markers p53 and Ki-67 and concluded that expression of p53 by more than 30% of cells was noted only in HGPUCa.[6,16]

This study has inherent limitations, as data on clinical outcome was not collected. Follow up if carried upon shall generate stronger evidence for the differences in pathologist opinion to grade urothelial neoplasm.

## Conclusion

The varied histology encountered in the present study suggests that papillary urothelial neoplasm should be considered as a heterogeneous disease with wide spectrum of biological and morphologic manifestations. We believe that it is prudent to dispatch consensus report of papillary urothelial neoplasm. In lieu of alarming disparity in opinion the imperative need of training of pathologists is reinforced. This study illustrates the importance of comprehensive, integrated and veritable pathological report with multiple opinions. The pathological report should also identify and specify prognostic features such as the grade, depth of tumor invasion and comment on presence of lamina propria and muscle in the specimen, which can direct molecular investigations and therapeutic decisions.

## References


1. Sharma P, Kini H, Pai RR, Sahu KK, Kini J. Study of the reproducibility of the 2004 World Health Organization classification of urothelial neoplasms. Indian J Pathol Microbiol 2015;58:59-61.
2. Miyamoto H, Brimo F, Schultz L, Huihui Y, Miller JS, Fajarado S. Low grade papillary urothelial carcinoma of the urinary bladder: A clinicopathological analysis of a post-World Health Organization/International Society of Urological Pathology Classification cohort from a single academic center. Arch Pathol Lab Med 2010;134:1160-3.
3. Grignon DJ. The current classification of urothelial neoplasms. Mod Pathol 2009;22:S60-9.
4. Montironi R, Beltran AL, Mazzucchelli R, Bostwick DG. Classification and grading of the non-invasive urothelial neoplasms: recent advances and controversies. J Clin Pathol 2003;56:91-5.
5. MacLennan GT, Kirkali Z, Cheng L. Histologic grading of noninvasive papillary urothelial neoplasms. Eur Urol 2007;51:889-98.
6. Cina SJ, Lancaster-Weiss KJ, Lecksell K, Epstein JI. Vorrelation of Ki-67 with the new World Health Organization/International Society of Urological Pathology Classification System for Urothelial neoplasia. Arch Pathol Lab Med 2001;125:646-51.
7. Oosterhuis JWA, Schapers RFM, Janssen-Heijnen MLG, Pauwels RPE, Newling DW, Kate F. Histological grading of papillary urothelial carcinoma of the bladder: prognostic value of the 1998 WHO/ISUP classification system and comparison with conventional grading systems. J Clin Pathol 2002;55:900-5.
8. Cheng L, MacLennan GT, Beltran AL. Histologic grading of urothelial carcinoma: a reappraisal. Hum Pathol 2012;43:2097-108.
9. Babjuk M, Oosterlinck W, Sylvester R, Kaasinen E, Bohle A, Palou-Redorta J, Roupret M. EAU guidelines on non-muscle-invasive urothelial carcinoma pf the bladder, the 2011 update. Eur Urol 2011;59:997-1008.
10. Eble JN. Non-invasive urothelial neoplasm. in: Eble JN, Sauter G, Epstein JI et al, (Eds.) World Health Organisation classification of tumors: pathology and genetics of tumors of the urinary system and male genital organs. IARC Press, Lyon(France); 2004: 26.
11. Papillary urothelial neoplasms and their precursors. In: Epstein JI, Amin MB, Reuter VE, eds. Bladder Biopsy Interpretation. Philadelphia: Lippincott Williams & Wilkins,2004.
12. Cheng L, Monitroni R, Davidson DD, Beltran AL. Staging and reporting of urothelial carcinoma of the urinary bladder. Mod Pathol 2009;22:S70-95.
13. Pasin E, Josephson DY, Mitra AP, Cote RJ, Stein JP. Superficial bladder cancer: an update on etiology, molecular development, classification and natural history. Rev Urol 2008;10:31-43.
14. Stepan AE, Dochit CM, Albita C, Simionescu C. The mechanisms of urothelial carcinogenesis: a literature review. Curr Health Sci J 2010;36:193-5.
15. Comperat E, Van der Kwast TH. Pathological staging of bladder cancer. Diagn Pathol 2013;19:366-74.

16. Yin H, Leong ASY. Histologic grading of noninvasive papillary urothelial tumors: validation of the 1998 WHO/ISUP system by immunophenotyping and follow-up. Am J Clin Pathol 2004;121:679-87.