DOI: 10.7860/NJLM/2024/69922.2876

Original Article

Pathology Section

Clinicomorphological Analysis of Urinary Bladder Lesions with Special Reference to Immunohistochemical Analysis of Grey Zone Lesions using Cytokeratin 20: A Cross-sectional Study

ANKITA SRIVASTAVA[1], RANI BANSAL[2], POOJA TREHAN[3], SANJAY PANDEY[4], ASHOK KUMAR SRIVASTAVA[5]

ABSTRACT

Introduction: There is a wide range of pathological lesions that can involve the urinary bladder, such as congenital anomalies, inflammatory conditions, metaplastic lesions and tumours which are responsible for significant morbidity and mortality worldwide. Broadly, bladder lesions are categorised as neoplastic and non neoplastic. Some urothelial lesions have mixed features or a tendency to transform into urothelial carcinoma such as metaplastic lesions, inverted papillomas, atypical papillary hyperplasia, Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP) and urothelial dysplasia. They are grouped as grey zone lesions. Identifying the malignant nature of grey zone lesions is difficult to identify by histopathological examination. Pathologists are trying to differentiate and classify bladder tumours using Immunohistochemical (IHC) markers such as Cytokeratin (CK) 20, CK-7, p53, Ki-67, etc., to help oncologists take proper decisions regarding patient management.

Aim: To study the histopathological lesions of urinary bladder tumours and diagnose the malignant nature of grey zone lesions using IHC marker CK 20.

Materials and Methods: This cross-sectional study of urinary bladder lesions was conducted in the Department of Pathology of Subharti Medical College, Meerut, Uttar Pradesh, India between December 2012 and October 2014. A total of 87 patients with urological complaints were selected for the study. Data related to age, sex, clinical symptoms and personal habits were noted on a working proforma. Biopsy materials were processed and stained with Haematoxylin and Eosin (H&E) for histopathological examination. The intensity of the reaction was determined in different fields, ranging from negative (0) to intense (3). Data thus obtained were analysed and presented in this paper.

Results: Out of 87 cases of urinary bladder lesions, 47 (54.02%) were neoplastic, 26 (29.88%) non neoplastic and 14 (16.09%) grey zone lesions. They were more common in males, particularly in the 7th and 6th decades of life, i.e., 27 (31.03%) and 22 (25.28%) respectively. The most common histopathological finding in non neoplastic lesions was inflammatory conditions seen in 21 (80.76%) cases; Papillary urothelial carcinoma-low-grade in 26 (55.31%) cases of neoplastic lesions and PUNLMP in 8 (57.14%) cases of grey zone lesions. The result was positive with CK 20 in 5 (62.5%) cases of PUNLMP, 1 (100%) urothelial dysplasia, and 1 (100%) poorly differentiated tumour tissues but negative in 3 (37.5%) cases of PUNLMP, 2 (100%) of Adenocarcinoma and 1 (100%) case of Nested Variant of Urothelial Carcinoma (NVUC).

Conclusion: CK 20 is a good IHC marker for determining the malignant nature of tissues in grey zone lesions. The results will be more reliable when used together with other markers.

Keywords: Haematuria, Metaplastic lesion, Neoplastic lesion

INTRODUCTION

Urinary bladder is a temporary muscular reservoir of urine lies in the anterior part of the pelvic cavity which gets empty through the urethra. Abnormal growths or tumours on the bladder lining are called bladder lesions. There is a wide range of pathological lesions that can involve the urinary bladder, including congenital anomalies, inflammatory conditions, traumatic injuries, metaplastic lesions and tumours. Neoplastic and non neoplastic lesions of the urinary bladder are collectively responsible for significant morbidity and mortality worldwide. Bladder cancer is the second most common malignancy of the genitourinary tract in males, following prostate cancer. It ranks as the fourth most common cancer in males and the ninth most common in females, accounting for 6% and 2% of total cancer incidences in men and women, respectively [1].

At large, the bladder lesions can be categorised as neoplastic or non neoplastic. Clinically and cystoscopically, some cases of cystitis may mimic neoplasms while certain flat, ulcerated neoplastic lesions may resemble inflammatory conditions. Papillary and polypoidal cystitis can be challenging to differentiate from papillary tumours of low malignant potential. Some urothelial lesions have mixed features or have tendency to transform into urothelial carcinoma, such as metaplastic lesions, inverted papillomas, atypical papillary hyperplasia, PUNLMP and urothelial dysplasia. These are grouped as grey zone lesions and determining their malignant nature through histopathological examination can be difficult. Pathologists around the world are trying to solve this issue and classify bladder tumours by non invasive detection methods using transcriptase-polymerase chain reaction and IHC stains such as CK 20, CK 7, p53, Ki-67, etc., so that oncologists can take proper decisions regarding patient management.

Among the known CKs, CK 19 is expressed in normal urothelial cells, whereas the recently identified CK 20 is expressed in urothelial carcinoma but not in normal urothelial cells [2]. CK 20 serves as a potential marker for bladder cancer and is significantly more sensitive than urinary cytology [3]. Antibodies to CK 20 can be used to identify a range of urothelial neoplasms arising from epithelium that normally contains the CK 20 protein.

The authors tried to find out histopathological lesions in diseases of the urinary bladder and the role of IHC marker CK 20 in differentiating the malignant nature of grey-zone lesions.

MATERIALS AND METHODS

The present study was a cross-sectional study conducted in the Department of Pathology, Subharti Medical College, Meerut, Uttar Pradesh, India. The study duration was from December 2012 to October 2014. The approval of the protocol was obtained by the Institutional Ethics Committee vide letter no. SMC/IEC/2012/211.

Inclusion criteria: Patients having urological complaints like haematuria, urinary retention, and lower abdominal pain were included in the study.

Exclusion criteria: Inadequate biopsies and crush artifacts were excluded from the study.

Study Procedure

Data related to age, sex, common clinical symptoms, family history, personal habits including tobacco smoking and/or chewing and findings from blood, urine and radiological investigations were noted on a working proforma. Bladder tissues were collected by biopsy, cystectomy or cystoprostatectomy for histopathological examination. A total of 87 cases were selected for analysis. Following the processing of the biopsy material, sections were stained with H&E and examined under a microscope. For the IHC study with CK 20, the paraffin blocks containing grey zone lesions were sent to "Lab Surgpath-Pathologist for the Human Protein Atlas" in Mumbai, Maharashtra, India.

In the study, 5-µm-thick deparaffinised and rehydrated sections were treated with antihuman mouse IgG CK 20 monoclonal antibodies at a dilution of 1:20 for 25 minutes and then exposed to a biotinylated secondary linking antibody for 25 minutes. At last, the slides were counterstained with Haematoxylin for one minute. The intensity of the reaction was determined in different fields, ranging from negative (grade 0) to superficial (umbrella cell) (grade 1), diffuse weak (grade 2), and intense (grade 3) [4].

STATISTICAL ANALYSIS

Data thus obtained were analysed and results were expressed in terms of frequency and percentage.

RESULTS

After histological examination of urinary bladder lesions, it was found that, in general, they were categorised as non neoplastic, neoplastic, or grey zone lesions. Out of a total of 87 cases, 26 (29.88%) were non neoplastic, 47 (54.02%) were neoplastic and 14 (16.09%) were grey-zone lesions.

Age-wise, the maximum number of patients, 27 (31.03%) belonged to the 61-70 years age group, followed by 22 (25.28%) patients aged 51-60 years. Only 5 (5.75%) patients were below 30 years of age. In terms of sex, the majority of the patients were male, with 69 (79.31%) out of a total of 87 cases. The male-female ratio was 3.8:1 [Table/Fig-1].

	Male	Female	
Age group (years)	n (%)	n (%)	n (%)
0-10	1 (1.15)	0	1 (1.15)
11-20	2 (2.30)	0	2 (2.30)
21-30	2 (2.30)	0	2 (2.30)
31-40	7 (8.04)	1 (1.15)	8 (9.19)
41-50	9 (10.34)	4 (4.59)	13 (14.94)
51-60	16 (18.39)	6 (6.89)	22 (25.28)
61-70	22 (25.28)	5 (5.74)	27 (31.03)
71-80	8 (9.19)	2 (2.30)	10 (11.5)
81-90	2 (2.30)	-	2 (2.30)
Total	69 (79.31)	18 (20.69)	87 (100.00)

[Table/Fig-1]: Age and sex-wise distribution of the patients of bladder lesions.

Personal habits: Smoking, tobacco chewing and alcohol consumption were found in 71 (81.6%) patients with bladder lesions. Among them, 17 (65.4%) cases of non neoplastic lesions, 40 (85.1%) cases of neoplastic lesions and all 14 (100%) cases of grey zone lesions had a habit of tobacco and/or alcohol.

The most common symptoms in patients with bladder lesions were haematuria alone or with urine retention, which was found in 56 (64.37%) patients, followed by urine retention in 19 (21.84%) cases and abdominal pain in 10 (11.49%) cases Bladder calculus was observed in 1 (1.15%) case [Table/Fig-2].

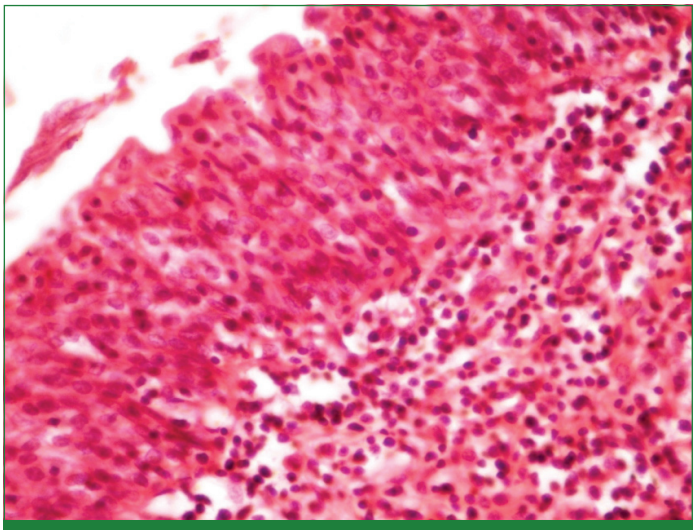
Urinary bladder symptoms	n (%)
Haematuria	37 (42.53)
Retention of urine	19 (21.84)
Hematuria and retention of urine both	16 (18.39)
Pain in abdomen	10 (11.49)
Haematuria and pain in abdomen	4 (4.59)
Calculus and retention of urine	1 (1.15)
Total	87 (100.00)

[Table/Fig-2]: Common symptoms in urinary bladder lesions.

Histopathological analysis of non neoplastic lesions: After histopathological examination of 26 cases of non neoplastic bladder lesions, 21 (80.77%) were inflammatory, 3 (11.54%) were congenital and 2 (7.69%) had normal morphology [Table/Fig-3]. Among the inflammatory lesions, chronic non specific cystitis [Table/Fig-4] was the most common seen in 11 (42.31%) cases, followed by eosinophilic cystitis in 4 (15.38%) cases and cystitis glandularis in 2 (7.69%) cases. Necrosed material was observed in one out of two cases with normal morphology.

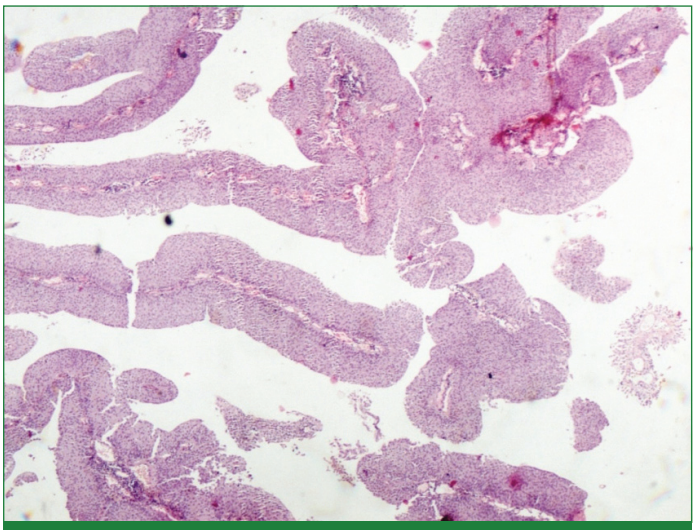
Type of non neoplastic lesions		n (%)
Inflammatory n=21 (80.77%)	Chronic non specific cystitis	11 (42.31)
	Eosinophilic cystitis	4 (15.38)
	Cystitis glandularis	2 (7.69)
	Acute on chronic cystitis	1 (3.84)
	Malakoplakia	1 (3.84)
	Ulcerative cystitis	1 (3.84)
	Interstitial cystitis	1 (3.84)
Congenital n=3 (11.54%)	Diverticulum	2 (7.69)
	Urachal cyst	1 (3.84)
Normal morphology n=2 (7.69%)	Normal tissue	1 (3.84)
	Normal tissue with necrosis	1 (3.84)
Total		26 (100.00)

[Table/Fig-3]: Spectrum of non neoplastic lesions.



[Table/Fig-4]: Photomicrograph of chronic non specific cystitis (H&E, 100x).

Histological analysis of neoplastic lesions: Out of 47 cases of neoplastic lesions, more than half, 26 (55.32%) were papillary urothelial carcinoma-low-grade [Table/Fig-5], followed by 15 (31.91%) cases of infiltrating urothelial carcinoma-high-grade and 2 (4.25%) cases of infiltrating urothelial carcinoma-low-grade [Table/Fig-6]. There was one (2.13%) case of Squamous cell carcinoma among the 47 neoplastic lesions.



[Table/Fig-5]: Photomicrograph of papillary urothelial carcinoma- low-grade (H&E, 40x).

Neoplastic	n (%)
Papillary urothelial carcinoma, low-grade	26 (55.32)
Infiltrating urothelial carcinoma, high-grade	15 (31.91)
Infiltrating urothelial carcinoma, low-grade	02 (4.25)
Infiltrating urothelial carcinoma with squamous differentiation	02 (4.25)
Urothelial papilloma	01 (2.13)
Squamous cell carcinoma	01 (2.13)
Total	47 (100.00)

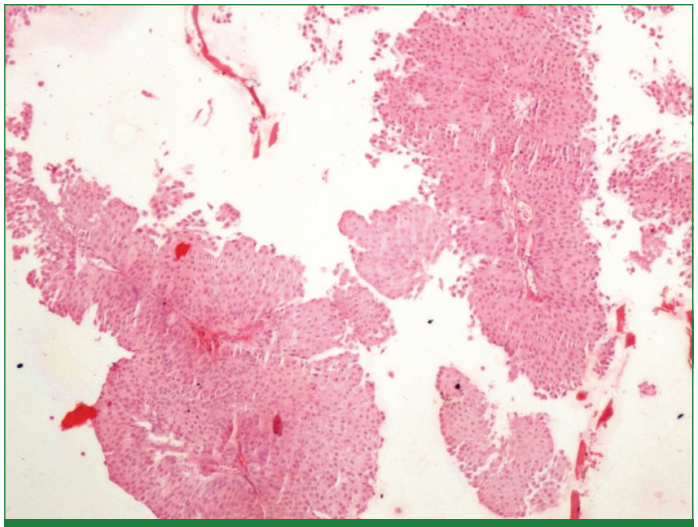
[Table/Fig-6]: Spectrum of neoplastic lesions.

Histological analysis of grey zone lesion: Out of 14 cases of grey zone lesions, 8 (57.14%) were PUNLMP. There are 2 (14.28%) cases of Nephrogenic adenoma and 1 (7.14%) case of NVUC [Table/Fig-7].

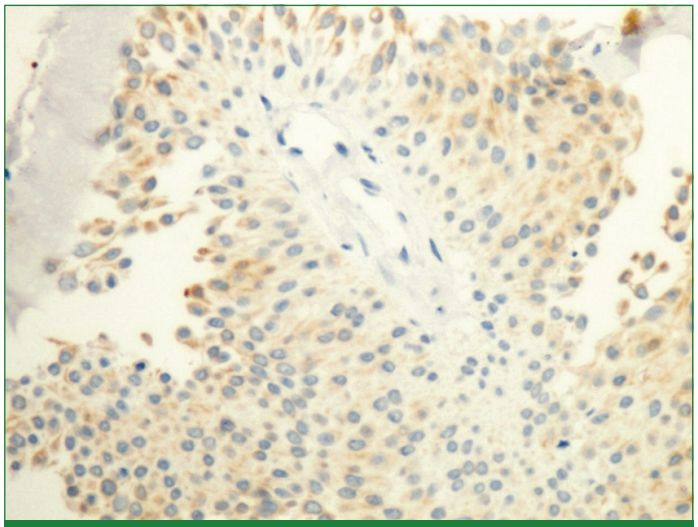
Grey-zone lesions	n (%)
Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP)	8 (57.14)
Nephrogenic adenoma	2 (14.28)
Nested Variant of Urothelial Carcinoma (NVUC)	1 (7.14)
Urothelial dysplasia	1 (7.14)
Papillary hyperplasia	1 (7.14)
Poorly differentiated tumour tissue	1 (7.14)
Total	14 (100.00)

[Table/Fig-7]: Spectrum of grey zone lesions.

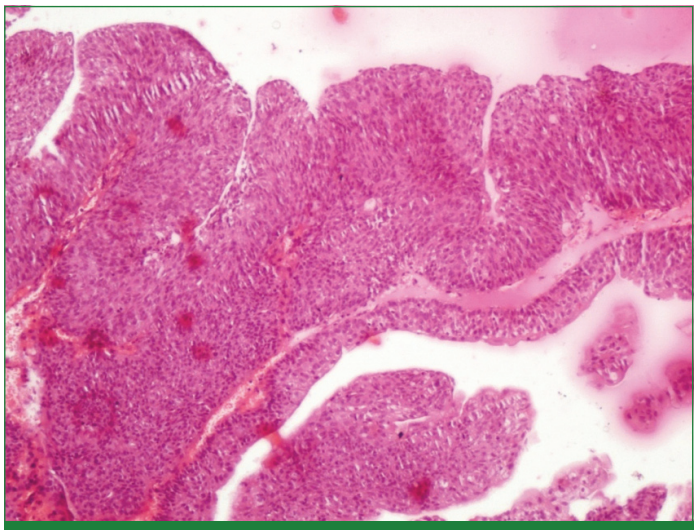
IHC analysis of grey zone lesions with CK 20: IHC with CK 20, out of eight cases of PUNLMP, 2 (25%) cases showed grade 2 (weak diffuse) positivity [Table/Fig-8,9], 3 (37.5%) showed grade 1 (superficial-umbrella) cell positivity [Table/Fig-10,11], and 3 (37.5%) were grade 0 (negative) [Table/Fig-12,13]. Two (100%) cases of nephrogenic adenoma and one (100%) case of NVUC showed negativity with CK20. One case of urothelial dysplasia revealed grade 2 (weak positivity) and 1 case of poorly differentiated tumour tissue showed grade 3 (strong positivity) with CK20 [Table/Fig-14-16].



[Table/Fig-8]: Photomicrograph of PUNLMP (H&E, 10x).



[Table/Fig-9]: Photomicrograph of PUNLMP (similar tumour as in [Table/Fig-8]) showing weak diffuse positivity, Grade 2 (CK 20 100x).

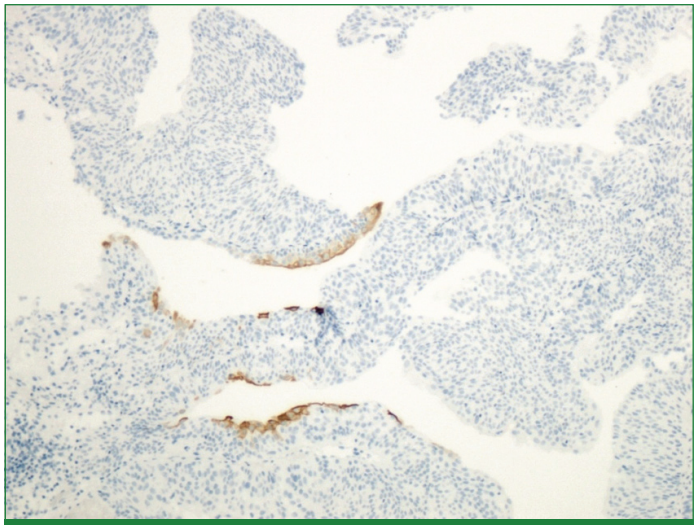


[Table/Fig-10]: Photomicrograph of PUNLMP (H&E, 40x).

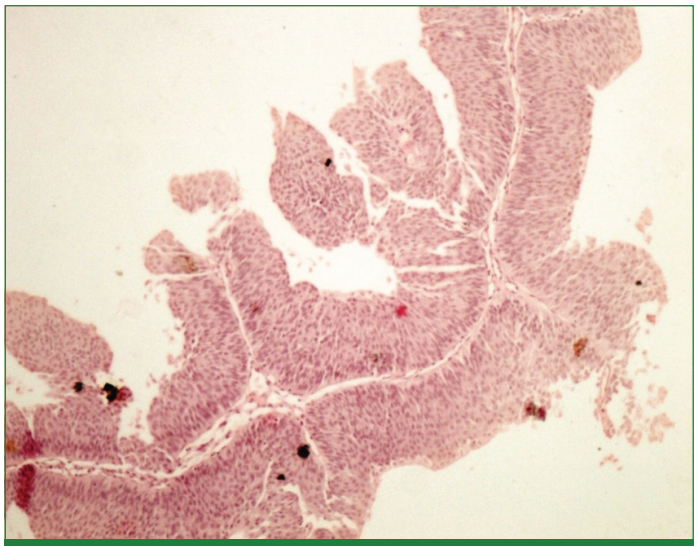
DISCUSSION

Urinary bladder lesions are abnormal growths or tumours present on the lining of the bladder. Broadly, they can be classified as neoplastic and non neoplastic lesions and are responsible for significant morbidity and mortality throughout the world. In this study, out of 87 cases of urinary bladder lesions, 61 (70.12%) were neoplastic (including grey-zone lesions) and 26 (29.88%) were non neoplastic, which was similar to the findings observed by Shrestha EP and Karmacharya K in Nepal, i.e., 79.25% and 20.75%, respectively [5].

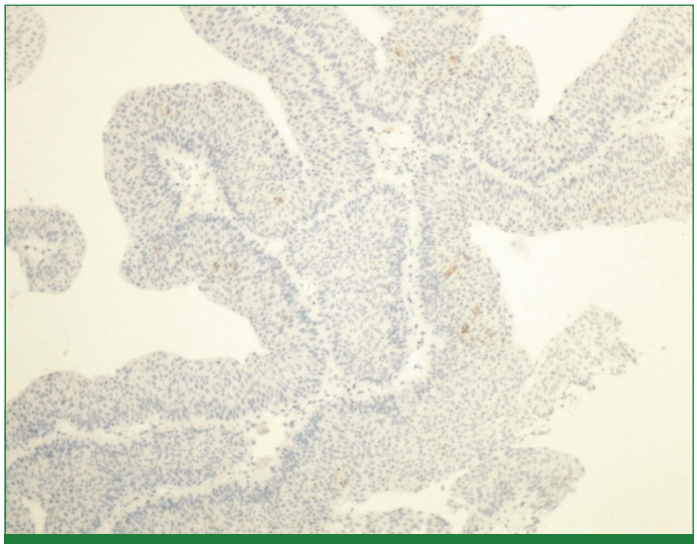
There was a marked predominance of male gender among patients with bladder lesions, with 69 (79.31%) males against 18 (20.69%) female amounting male-female ratio of 3.8:1. This finding was consistent with the findings observed by Ploeg M et al., (4:1), Mumtaz S et al., (4.3:1), and Joshi HN et al., (3.66:1) [6-8]. However,



[Table/Fig-11]: Photomicrograph of PUNLMP (similar tumour as in [Table/Fig-9]) showing superficial positivity Grade 1 (CK 20 40x).



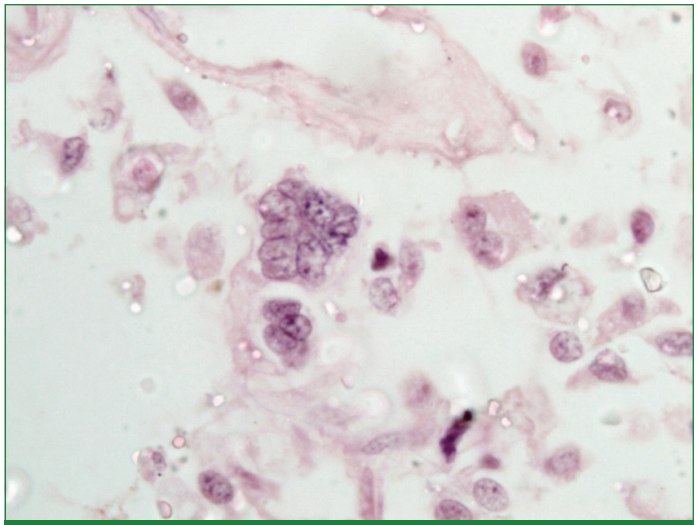
[Table/Fig-12]: Photomicrograph of PUNLMP (H&E, 40x).



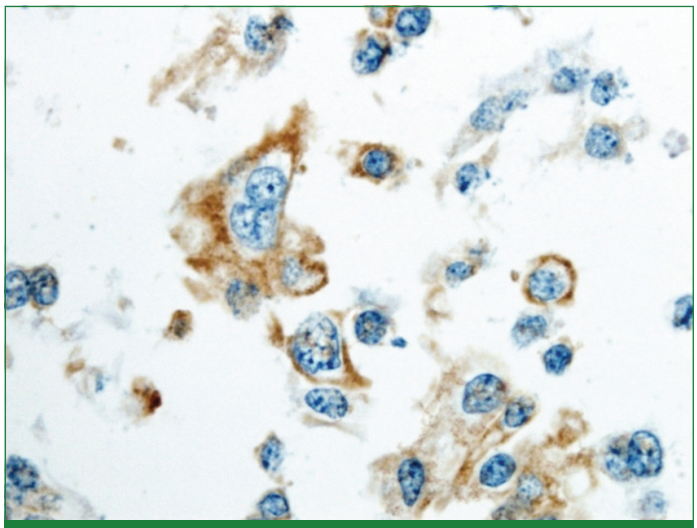
[Table/Fig-13]: Photomicrograph of PUNLMP (similar tumour as in [Table/Fig-12]) showing negativity, Grade 0 (CK 20 40x).

the male-female ratio was lower in a study conducted by Hasan SM and Imtiaz F (2.58:1) and higher in studies by Matalka I et al., (9:1) and Agarwal S et al., (15.7:1) [9-11].

Age-wise, the maximum number of bladder lesions was seen in the 61-70 age group, with 27 (31.03%) cases, followed by 22 (25.28%) in the 51-60 age group. Similar findings were observed by Srikousthubha S et al., Shah A et al., and Vaidya S et al., [1,12,13].



[Table/Fig-14]: Photomicrograph showing poorly differentiated tumour cells (H&E, 100x).



[Table/Fig-15]: Photomicrograph of Poorly differentiated tumour cells as in [Table/Fig-14] showing strong positivity, Grade 3 (CK 20 100x).

Grey zone lesions (Total 14)	Number	Expression of CK 20	
PUNLMP n=8 (57.1%)	02	Weak positive	2
	03	Superficial cells positive	1
	03	Negative	0
Nephrogenic adenoma	02	Negative	0
NVUC	01	Negative	0
Urothelial dysplasia	01	Weak positive	2
Papillary hyperplasia	01	Negative	0
Poorly differentiated tumour tissue	01	Positive	1

[Table/Fig-16]: Expression of CK 20 in grey zone lesions.

History of tobacco consumption (smoking and/or chewing) was seen in 17 (65.38%) cases of non neoplastic lesions, 40 (85.11%) cases of neoplastic lesions and 14 (100%) cases of grey zone lesions. Similarly, smoking was identified as the most significant risk factor in a study by Joshi HN et al., where 78% of patients had a history of smoking [8]. Pashos CL et al., reported tobacco smoking in 65.3% of bladder malignancy cases [14].

Haematuria and urinary retention, or both were the main presenting symptoms in 56 (64.37%) and 36 (41.38%) cases of bladder diseases, respectively. This finding was consistent with the observations of Messing EM et al., and Shepherd EA et al., [15,16], who reported haematuria as the chief presenting symptom of urothelial malignancy in their studies. Agarwal S et al., reported haematuria in 84% of cases and Ray et al., reported it in 91% of urinary bladder tumour cases [11,17].

The most common histopathological feature was inflammatory conditions in non neoplastic lesions, seen in 21 (80.77%) cases; low-grade papillary urothelial carcinoma in neoplastic lesions found in 26 (55.32%) cases; and PUNLMP in grey zone lesions, seen in 8 (57.14%) cases of bladder lesions. In another study Agarwal S et al., reported similar findings [11].

The expression of CK 20 in grey zone lesions varied from strong positive to negative. Out of eight cases of PUNLMP, 2 (25%) cases showed weak positivity, 3 (37.5%) showed positivity in the superficial (umbrella) cells and 3 (37.5%) were negative. These findings were consistent with the findings of other pathologists, Arias-Stella JA et al., and Raheem SA et al., who have noted that the expression of CK 20 is highly heterogeneous in tumour cells [18,19]. Some of them showed positivity in the superficial layer of cells, while others show diffuse weak positivity. Barbisan F et al., reported a superficial staining pattern in 53.7% of cases of PUNLMP and Yildiz IZ et al., reported positivity in the full thickness of the urothelium in 30% of cases, 2/3 of thickness in 60% of cases and in the upper 1/3 in 10% of cases [20,21].

Limitation(s)

The main limitation of this study was the lower number of cases in few varieties of urinary bladder lesions. Due to the diverse expression of CK 20 in the same type of tumours, in some cases, the use of multiple IHC markers/CKs is suggested in such studies.

CONCLUSION(S)

Urinary bladder lesions are frequently seen in elderly males. Haematuria and retention of urine or both are the most common presenting symptoms. The majority of urinary bladder lesions are malignant in nature, with papillary urothelial carcinoma low-grade being the most common in neoplastic lesions and PUNLMP in grey zone lesions. The effect of CK 20 varies from strong positive to negative not only in different tumours of grey zone lesions but also in the same type of tumours. Therefore, it is advised that the study should be conducted with multiple CKs like CK 7, CD 44, GATA3 on a large number of cases.

REFERENCES

[1] Srikousthubha S, Raghuveer CV, Hungle S. Profile of lesions in cystoscopic bladder biopsies: A histopathological study. J Clin Diagn Res. 2013;7(8):1609-12.
[2] Klein A, Zemer R, Buchumensky V, Klaper R, Nissenkorn I. Expression of cytokeratin 20 in urinary cytology of patients with bladder carcinoma. Cancer. 1998;82(2):349-54.
[3] Buchumensky V, Klein A, Zemer R, Kessler OJ, Zimlichman S, Nissenkorn I. Cytokeratin 20: A new marker for early detection of bladder cell carcinoma. J Urol. 1998;160(6 Pt 1):1971-74.
[4] Popov H, Stoyanov GS, Ghenev P. Role of Cytokeratine 20 as a predictive and prognostic marker in urothelial neoplasms. Cureus. 2022;14(11):e31607.
[5] Shrestha EP, Karmacharya K. Profile of histopathological lesions of urinary bladder: A five-year study. J Pathol Nepal. 2016;6:1001-04.
[6] Ploeg M, Aben KKH, Kiemeney LA. The present and future burden of urinary bladder cancer in the world. World J Urol. 2009;27(3):289-93.
[7] Mumtaz S, Hashmi AA, Hasan SH, Edhi MM, Khan M. Diagnostic utility of p53 & CK20 Immuno-histochemical expression grading Urothelial malignancy. Int Arch of Med. 2014;7(1):36.
[8] Joshi HN, Makaju R, Karmacharya A, Karmacharya RM, Shrestha B, Shrestha R, et al. Urinary bladder carcinoma: Impact of smoking, age and its clinico-pathological spectrum. Kathmandu Univ Med J (KUMJ). 2013;11(44):292-95. Doi: 10.3126/kumj.v11i4.12524.
[9] Hasan SM, Imtiaz F, Hasan SM. Frequency of transitional cell carcinoma in local suburban population of Karachi. JLUMHS. 2007;6(2):83-85.
[10] Matalka I, Bani-Hani K, Shotar A, Bani Hani O, Bani Hani I. Transitional cell carcinoma of the urinary bladder: A clinicopathological study. Singapore Med J. 2008;49(10):790-94.
[11] Agarwal S, Pandey P, Ralli M, Agarwal R, Yadav A, Dwivedi N. A clinicopathologic study of urinary bladder lesions amongst North Indian population: An experience from a tertiary care centre. Cureus. 2024;16(5):e59792.
[12] Shah A, Srivastava M, Samdurkar A, Sigdel G. Spectrum of lesions in urinary bladder- A histopathological study. J Uni College Med Sci. 2018;06(02):24-27.
[13] Vaidya S, Lakhey M, Sabira KC, Hirachand S. Urothelial tumours of the urinary bladder: A histopathological study of cystoscopic biopsies. J Nepal Med Assoc. 2013;52(191):575-78.
[14] Pashos CL, Botteman MF, Laskin BL. Bladder cancer: Epidemiology, diagnosis and management. Cancer Pract. 2002;10(6):311-22.
[15] Messing EM, Young TB, Hunt VB, Gilchrist KW, Newton MA, Bram LL, et al. Comparison of bladder cancer outcome in men undergoing hematuria home screening versus those with standard clinical presentations. J Urology. 1995;45(3):387-97.
[16] Shepherd EA, Stapley S, Neal RD, Rose P, Walter FM, Hamilton WT. Clinical features of bladder cancer in primary care. Br J Gen Pract. 2012;62(602):598-604.
[17] Ray D, Mondal R, Achryya S, De S, Mondal S. A retrospective study of bladder cancer and the impact of age, sex and smoking habits with related clinicopathological correlations in the tribal population of Bankura, WB, India. OISR J Dent Med Sci. 2013;10(4):29-32.
[18] Arias-Stella JA 3rd, Shah AB, Gupta NS, Williamson SR. CK20 and p53 immunohistochemical staining patterns in urinary bladder specimens with equivocal atypia. Arch Pathol Lab Med. 2018;142(1):64-69.
[19] Raheem SA, Saied AN, Al Shaer R, Mustafa O, Ali AH. The role of CK20, p53 and p63 in differentiation of some urothelial lesions of urinary bladder, immune-histochemical study. Open J Pathol. 2014;4(4):181-93.
[20] Barbisan F, Santinelli A, Mazzucchelli R, Lopez-Beltran A, Cheng L, Scarpelli M, et al. Strong immunohistochemical expression of fibroblast growth factor receptor 3, superficial staining patter of cytokeratin 20 and low proliferative activity define those papillary urothelial neoplasms of low malignant potential that do not recur. Cancer. 2008;112 (3):636-44.
[21] Yildiz IZ, Recavarren R, Armah HB, Bastacky S, Dhir R, Parwani AV. Utility of a dual immunostain cocktail comprising of p53 and CK20 to aid in the diagnosis of non-neoplastic and neoplastic bladder biopsies. Diagn Pathol. 2009;4:35.

PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Pathology, T. S. Misra Medical College and Hospital, Lucknow, Uttar Pradesh, India.
2. Professor and Head, Department of Pathology, Subharti Medical College, Meerut, Uttar Pradesh, India.
3. Assistant Professor, Department of Pathology, T. S. Misra Medical College and Hospital, Lucknow, Uttar Pradesh, India.
4. Professor, Department of General Surgery, Subharti Medical College, Meerut, Uttar Pradesh, India.
5. Dean and Professor, Department of Forensic Medicine, T. S. Misra Medical College and Hospital, Lucknow, Uttar Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Ankita Srivastava,
Associate Professor, Department of Pathology, T. S. Misra Medical College and Hospital, Lucknow-226008, Uttar Pradesh, India.
E-mail: drankitapatho@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Feb 04, 2024
- Manual Googling: Jun 11, 2024
- iThenticate Software: Jul 10, 2024 (13%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Feb 03, 2024**
Date of Peer Review: **Mar 06, 2024**
Date of Acceptance: **Jul 11, 2024**
Date of Publishing: **Oct 01, 2024**