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A Histomorphological Study of Urinary Bladder Lesions in a Tertiary Care Hospital

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Abstract

Urinary bladder lesions, both benign and malignant, are becoming more common in modern times. The urinary bladder is susceptible to a range of lesions, including benign tumors, malignant tumors, and non-neoplastic lesions. A lot of people either die or go through a lot of pain because of neoplastic bladder lesions. Understanding bladder tumors involve exploring their risk factors, which include smoking, occupational exposures, and chronic bladder irritation. In terms of genitourinary cancers, the most common ones are prostate and urinary bladder cancers. Bladder cancers are curable if diagnosed at an early stage. Cystoscopy to see the bladder mucosa and biopsy of any suspicious lesion and TURBT (transurethral resection of the bladder tumour) samples with histological evaluation are the most reliable methods for cancer diagnosis. Early and accurate diagnosis is crucial for effective management and improved patient outcomes. This case series presents cases with different urinary bladder lesions encountered in our tertiary referral hospital, providing insights into their presentation, diagnostic challenges, role of histopathology in making a diagnosis and thereby helping the clinician in exploring treatment options.

Keywords: *Benign, Bladder, Genitourinary, Histopathology, Malignant, Transurethral.*

Introduction

Bladder lesions, whether non-neoplastic or malignant, may be very debilitating [1]. Urinary bladder cancer is a major killer among humans, accounting for an estimated 200,000 deaths and 5,00,000 new cases annually worldwide [2]. It is also one of the most common malignancies. Urinary bladder lesions are diverse ranging from benign lesions to frank malignancies. Cystitis, malakoplakia, and Tuberculosis are examples of non-neoplastic lesions. Most often, Clinical symptoms like painful micturition are caused by cystitis, which is one of the most prevalent bladder lesions. The sixth decade of life has a preponderance for malignant tumours compared to benign ones [3]. Neoplastic lesions cause a lot of sickness and death all around the globe. Urothelial carcinoma accounts for 90% of primary bladder tumours and is the most prevalent malignant tumour in this group [4]. It ranks sixth globally in incidence. Neoplastic lesions cause a lot of sickness and death all around the globe. Among cancers affecting the genitourinary tract, urinary bladder cancer ranks second, just behind prostate cancer [5]. Patients suspected of having bladder tumours are often diagnosed using cystoscopic guided biopsies in conjunction with histopathological examination [6,7]. Cancer of the bladder is more likely among people who smoke, who are exposed to specific chemicals at work, who experience urinary tract infections, who have a personal or family history of the disease, and who have undergone radiation

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therapy. The combination of TURBT (Transurethral Resection of Bladder Tumor) and intravesical BCG(Bacillus Calmette-Guérin) therapy is recommended for managing bladder carcinomas. Early diagnosis and effective management of urinary bladder carcinoma is crucial for improving patient outcomes, given the disease's propensity for recurrence and progression. Therefore, this study is set out to provide histological evidence of the variety of urinary bladder lesions identified by TURBT and cystoscopic biopsies.

Materials and Methods

Study Design

A cross-sectional study was done.

Study Population

The study was conducted in the Urology department and Department of pathology, Sree Balaji Medical College and Hospital from January 2023 to February 2024. After the patients gave their verbal and written informed permission, clinical information, cystoscopic findings, clinical diagnoses of all cases of urinary bladder lesions were obtained after they visited the urology department and their cystoscopic and Transurethral Resection of Urinary Bladder Tumors(TURBT) specimens were forwarded to the histopathology lab.

Inclusion Criteria

Patients attending the urology department were included in the study after attaining verbal and written consent. Their clinical presentation and details were obtained. Questions about personal habits like smoking and alcohol were included. The cystoscopic findings, cystoscopic biopsies and TURBT specimens were forwarded to histopathology lab for processing and histopathological examination.

Exclusion Criteria

Patients who were not willing to participate were excluded from the study. Inadequate biopsies due to inadequate tissue content, autolyzed tissue, specimen not sent in formalin, biopsies in which definitive histopathological opinion was deferred were excluded from the study.

Study Procedure

The specimens were fixed in 10% formalin overnight. Grossing of formalin fixed specimens were done as per CAP protocol for cystectomy specimens and representative sections were taken. Small biopsies of TURBT were all embedded. Five microns thick serial sections were routinely processed, stained with Hematoxylin & Eosin, and examined microscopically. Immunohistochemistry with three markers GATA3, CK5 and CK20 were done for indicated cases for definitive diagnosis. Reporting of neoplastic lesions were done according to 2022 WHO classification of urogenital tumors, 5^{th} edition, staging as per 8^{th} edition of American Joint Committee on Cancer (AJCC) and final report in CAP protocol for examination of cystectomy specimens from patients with carcinoma of the urinary bladder.

Sample Size

The total sample size is 384. The sample size is calculated using the sample size formula for categorical data derived from the principles of statistical estimation and confidence intervals.

$$n = \frac{Z^2\, p\, (1-p)}{E^2}$$

where:

n = sample size

Z = Z-score corresponding to the confidence level 95% =1.96

p = expected proportion, 0.5 because of unknown patient population

E = margin of error (0.05, for a ±5% margin of error)

$$n = \frac{(1.96)^2\ 0.5\ (1-0.5)}{(0.05)^2}$$

$$n = \frac{3.8416 * 0.25}{0.0025}$$

$$n = \frac{0.9604}{0.0025}$$

n ≈ 384

The total sample size was 384 patients who attended the urology department during the one year period from January 2023 to February 2024.

Statistical Analysis

The whole data were entered in MS-excel master sheet and simple univariate and multivariate analysis was done with the help of Statistical Package for Social Sciences (SPSS) IBM version 25.0 software for windows to calculate the age and sex distribution of bladder lesions. Calculation of mean values, Standard deviation and percentages were done. Categorical variables were summarised as percentages. For all the analyses, the confidence interval was 95%, with a level of error of 0.05%. The data is presented as tables.

Results

In the present study, during the 1 year duration from January 2023 to February 2024, we received 384 bladder specimens which included 58 cystoscopic biopsies and 326 TURBT specimens. The age group ranged from fifteen to eighty years old. 280 patients were males and 104 patients were females (Table 1). Majority of benign lesions were seen in the 4th decade, while malignant tumors were more common in the 6th decade and seen in males when compared to females. Out of the 384 bladder lesions, non-neoplastic lesions comprised 69 cases (18%) and neoplasms were 315 cases(82%). Most common symptom among all these patients was painless hematuria (Table 2). 218 patients (57%) diagnosed with malignancy suffered from unexplained weight loss. Lesser common symptoms included fever, nocturia and abdominal pain. The most common cystoscopic finding in non-neoplastic lesion was thickened and red bladder mucosa with friable areas and haemorrhage. The common cystoscopic finding in neoplastic lesion was grey white friable soft tissue growth which varied in size from being small to involving the entire mucosa and extending to deeper tissues.

Table 1. Gender and Age Wise Distribution of Benign and Malignant Tumors

Age (years)	**Non-neoplastic lesions**		**Total no (%)**	**Neoplastic lesions**		**Total no (%)**
	Male	**Female**		**Male**	**Female**	
<20 years	4	2	6(9%)	0	0	0
21-30 years	1	2	3(4%)	10	4	14(1 %)
31-40 years	9	4	13(19%)	19	5	24(8%)
41-50 years	6	3	9(13%)	63	34	97(31%)
51-60 years	13	7	20(29%)	108	18	126(40%)
61-70 years	6	4	10(14%)	27	13	40(13%)
>70 years	4	4	8(12%)	10	04	14(4%)
	43	26	69(100%)	237	78	315(100%)
MEAN AGE ± SD	47.8 ± 8.06			MEAN AGE ± SD	51.5±7.19	
Age range	15-78 years			Age range	21-78 years	

Table 2. Distribution According to Clinical Features

S.No	Clinical Features	No of Cases	%
1	Hematuria	265	69
2	Dysuria	144	14
3	Increased frequency	105	27
4	Urgency	3	2
5	Abdominal pain	3	2
6	Incomplete voiding	117	30
7	Fever	58	15
8	Weight loss	218	57
9	Nocturia	30	8

Out of the 69 non-neoplastic lesions, chronic cystitis was the commonest lesion comprising 53 cases representing 77% of non-neoplastic lesions, other non-neoplastic lesions were granulomatous cystitis (7 cases, 10%) charecterized by presence of epithelioid granulomas in the bladder tissue on microscopic examination(Figure 1), endometriosis(4 cases, 7%), urachal cyst and malakoplakia one case each (1%). In malakoplakia, sheets of eosinophilic histiocytes were seen on microscopy. The variants of benign histology included cystitis glandularis 3 cases (4%) (Table 3). These results were in line with those of investigations on chronic non-specific cystitis by Shruthi HP et al[4] and Srikoustubha et al [9] who discussed that Non-invasive benign lesions were found to be more prevalent compared to Invasive Urothelial Carcinomas.

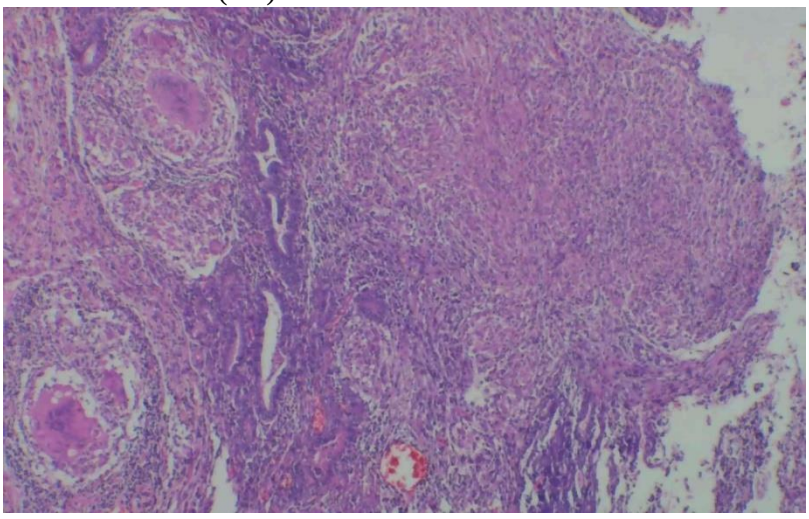

Figure 1. Presence of Epithelioid Granulomas with Langhan's Type of Giant Cells

Table 3. Histopathological Spectrum of Non-neoplastic Lesions

Type of lesion	No of cases	%
Chronic non-specific Cystitis	53	77
Granulomatous cystitis	7	10
Endometriosis	4	7
Urachal cyst	1	1
Malakoplakia	1	1
Cystitis glandularis	3	4
TOTAL	69	100

In one of the benign lesions with bladder endometriosis, the patient was a 36 year old female who came with 6 month old history of pelvic pain, dysuria and intermittent hematuria which worsened during menstruation. Imaging studies and cystoscopy revealed a bladder lesion suspicious for endometriosis. Patient underwent laproscopic excision of endometriotic lesions with partial cystectomy. Histopathology revealed presence of endometrial glands and stroma in the bladder tissue lined by transitional epithelium (Fig. 2).

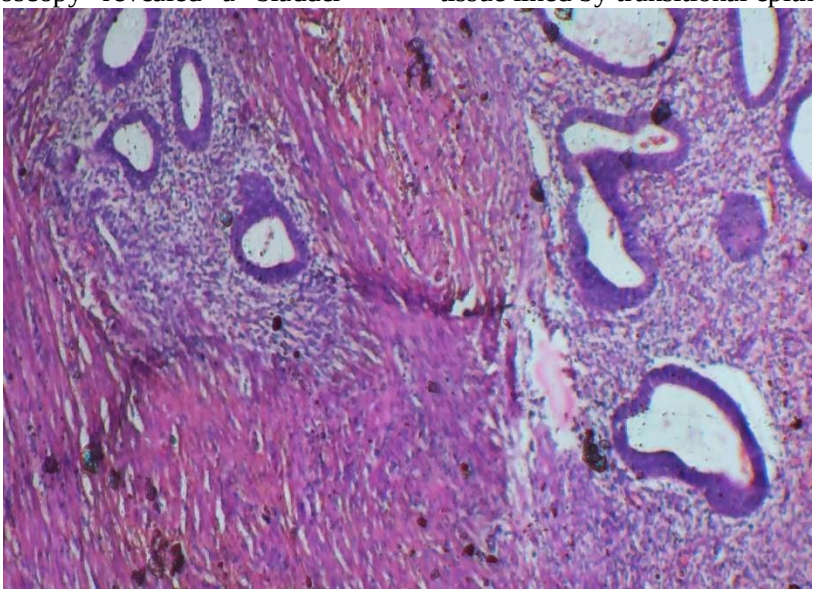

Figure 2. Endometrial Glands and Stromal Cells in Bladder Tissue on H&E Section

Table 4. Histopathological Spectrum of Neoplastic Lesions

Type of Lesion	**No of Cases**	**%**
Papillary Urothelial neoplasm of Low Malignant Potential	92	29
Papillary Urothelial Carcinoma-Low Grade	108	34
Papillary Urothelial Carcinoma-High Grade	101	32
Invasive Urothelial Carcinoma	14	5
TOTAL	315	100

The majority of the malignant neoplasms (76% of the total) were located in the 51-80 age category, and there were 70% male cases (a male-to-female ratio of 2.2:1). All the male patients were chronic smokers (Table 4).

Out of all the 315 neoplastic lesions, the most common type was urothelial carcinoma, which included 92 cases (29%) of Papillary Urothelial Neoplasm of Low Malignant Potential(PUNLMP), Papillary Urothelial Carcinoma-Low Grade were 108 cases (34%) and High Grade were 101 cases(32%). 14 cases (5%) were diagnosed with Invasive Urothelial Carcinomas. 4 cases of Invasive Urothelial Carcinomas showed squamous differentiation. Out of the 14 cases of Invasive Urothelial Carcinoma, one case showed clear cell urothelial carcinoma. These results were consistent with those of Laishram et al. [11], who found that 53.8% of the lesions were non-invasive papillary urothelial carcinomas, 15.38% of superficially invasive bladder carcinomas, and 30.77 % of muscle invasive carcinomas.

Of the 92 cases which were reported as Papillary Urothelial Neoplasm of Low Malignant Potential, microscopy showed thicker epithelial lining with hypercellularity and preserved polarity of cells with no atypia, rare/no mitosis was seen. Urothelial cells were all uniform with only slight nuclear enlargement (Figure 3).

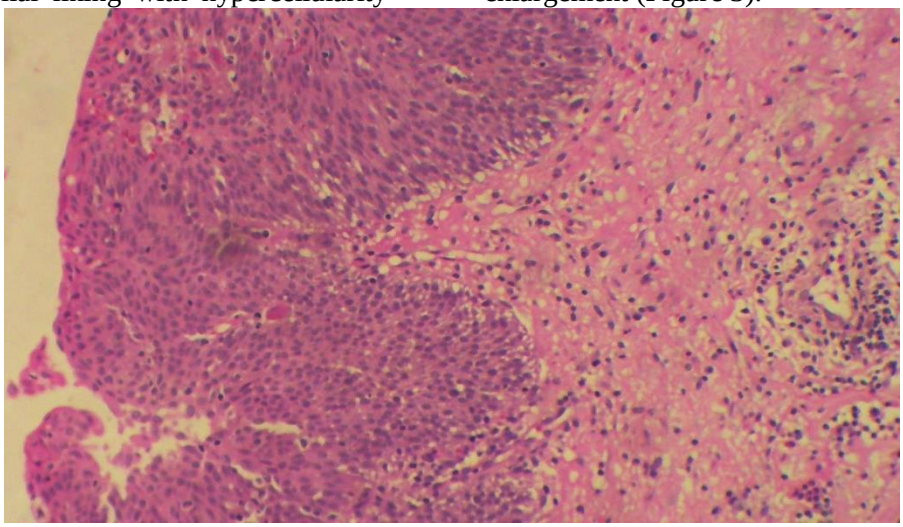

Figure 3. Thick Hypercellular Uniform Urothelial Lining with No Breach

108 cases were diagnosed with Papillary Urothelial Carcinoma-Low Grade based on routine histopathology and staining with GATA3, CK5 and CK20 on Immunohistochemistry. H&E showed neoplastic papillary branching of urothelial lining with cells showing slight variation with minimal loss of polarity (Figure 4) staining with CK 5 was negative (Figure 5). CK 20 showed diffuse positivity of the neoplastic urothelial lining(Figure 6). However, IHC plays a less significant role in making a diagnosis of Papillary Urothelial Carcinoma-Low Grade.

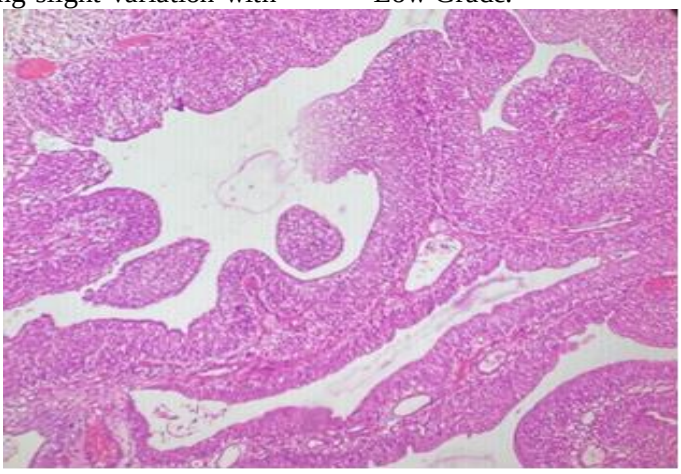

Figure 4. Papillary Architecture of Neoplastic Urothelial Lining

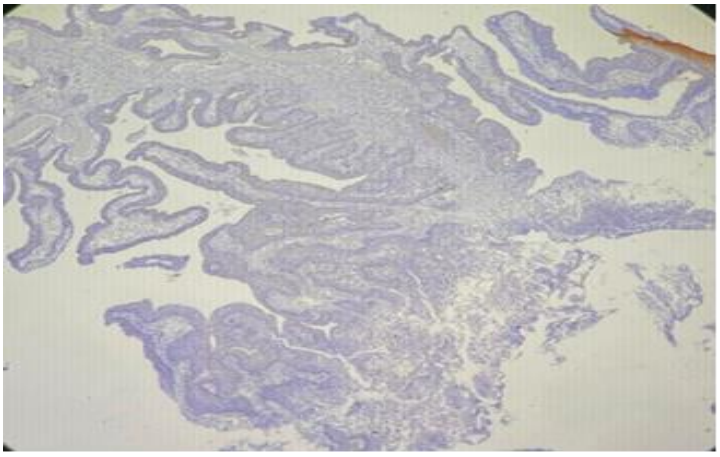

Figure 5. IHC staining for CK5 was Negative

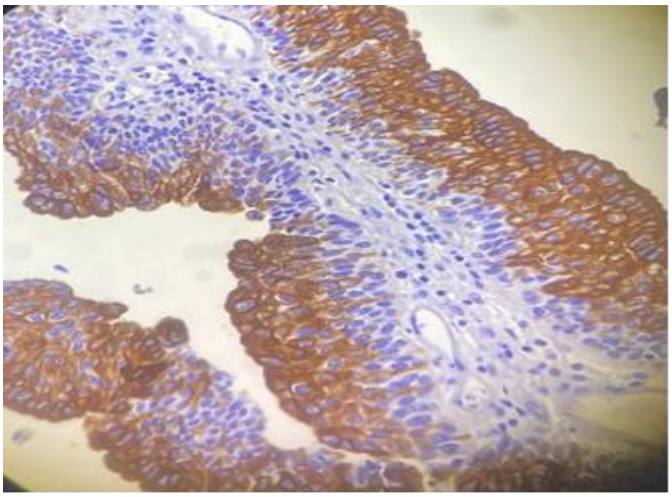

Figure 6. CK 20 showing Diffuse Positivity of Neoplastic Lining Cells

101 cases were diagnosed with Papillary Urothelial Carcinoma-High Grade on histopathology and IHC positive staining with GATA3 (Figure 8) and CK20 (Figure 9) and negative for CK5 (Figure 10). Histopathology showed exophytic tumors in papillary pattern with atypical cells showing loss of polarity and evident nuclear pleomorphism (Figure 7).

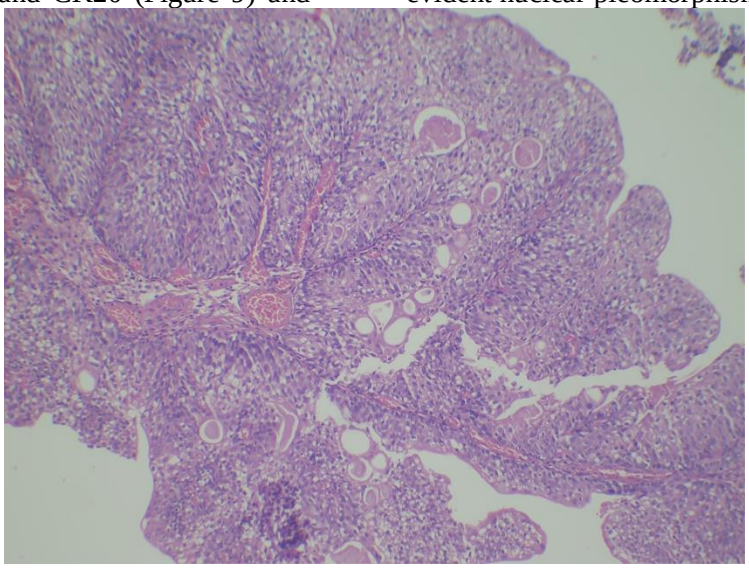

Figure 7. Papillary Urothelial Carcinoma-High Grade.

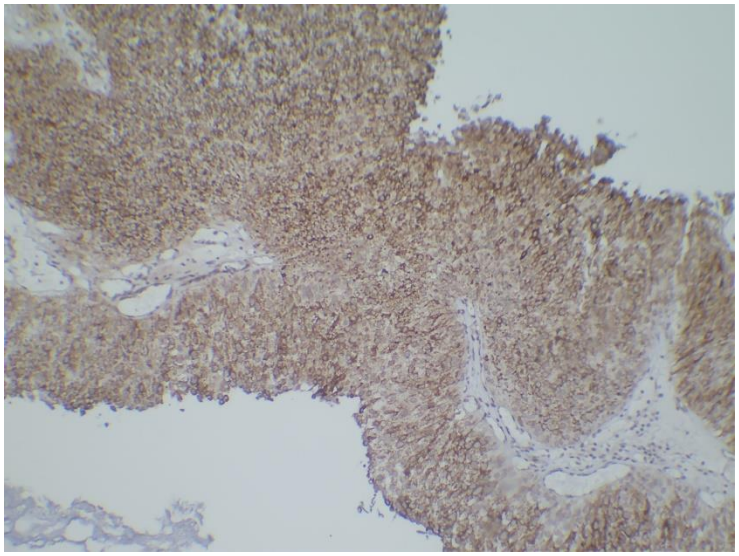

Figure 8. Diffuse Positivity for GATA3 in Papillary Urothelial Carcinoma-High Grade

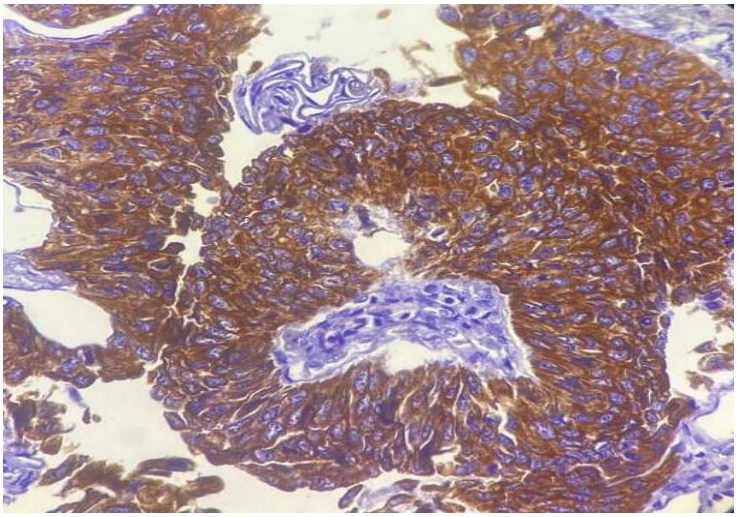

Figure 9. Diffuse Positivity for CK20 in Papillary Urothelial Carcinoma-High Grade

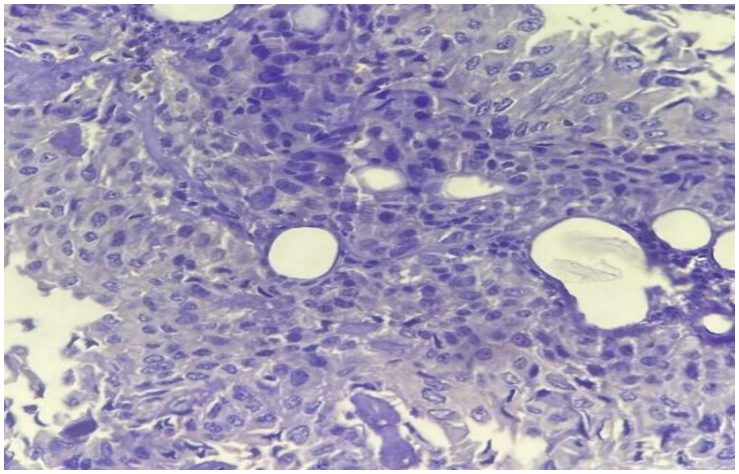

Figure 10. IHC Negative Staining for CK5

14 cases (5%) diagnosed with Invasive urothelial carcinomas showed nests, sheets and individual tumor cells with marked pleomorphism invading into the muscularis propria (Figure 11). IHC showed positivity for GATA3 (Figure 12) and CK5 (Figure 13) with

negativity for CK20. 4 cases of invasive urothelial carcinomas showed squamous differentiation with intercellular bridges or keratinization (Figure 14). Out of the 14 cases of Invasive Urothelial cCarcinomas, one case showed clear cell urothelial carcinoma with sheets of clear cells with defined cell membranes and clear cytoplasm (Figure 15).

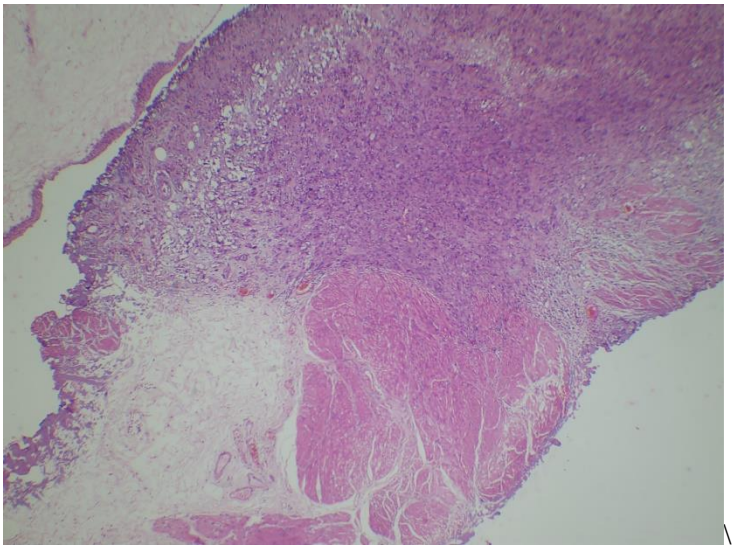

Figure 11. Tumor Cells Invading Muscularis Propria

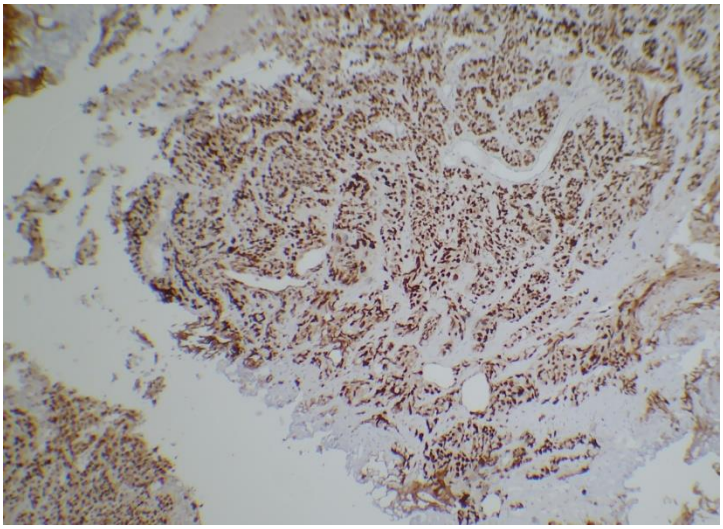

Figure 12. IHC showing Diffuse Positivity for GATA3

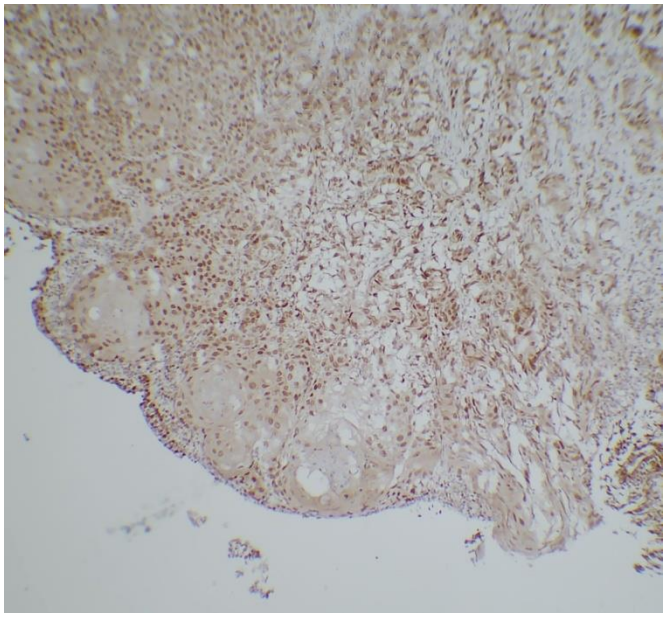

Figure 13. IHC showing Strong Positivity for CK5

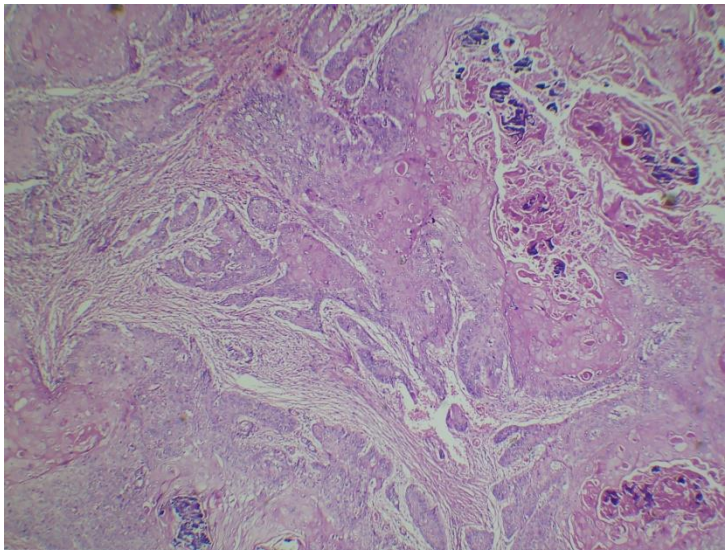

Figure 14. Islands of Tumor Cells with Keratinisation

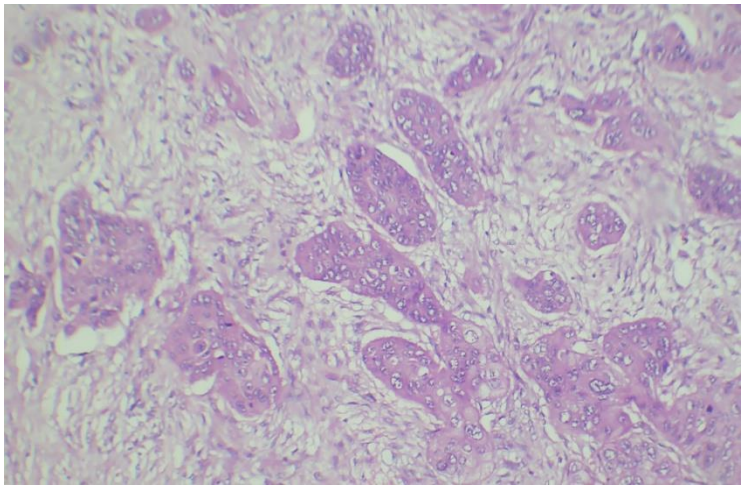

Figure 15. Invasive Urothelial Carcinoma with Islands of Clear Cells

Discussion

The primary objective of this study is to emphasize the crucial role of histopathological examination in diagnosing urinary bladder lesions. Various modes of diagnosis including cystoscopic imaging, urine cytology and biopsy with histopathological examination aided by ancillary testing like IHC are useful in identifying bladder lesions. Each of these diagnostic methods has inherent limitations and may not accurately detect bladder tumors in every instance.

In the present study of 1 year duration, out of 384 bladder specimens, 315 were malignant tumors accounting for 82% of all bladder biopsies which accounted for high mortality and morbidity and non-neoplastic lesions were 69(18%) [4]. Urologists may see the whole bladder mucosa on cystoscopy and take tissue samples from tumours for histopathological examination using transurethral balloon biopsy (TURBT), which is the gold standard in diagnostics and treatment [4,5]. All these diagnostic methods cannot be used to diagnose bladder tumors due to their own limitations.

Ninety percent of bladder malignancies are urothelial carcinomas, with squamous and adenocarcinomas following closely behind. Invasive urothelial carcinomas can be classified into two main kinds: non-muscle invasive bladder carcinoma and muscle invasive carcinoma.

Age groups 51–60 years(30%), 61–70 years(13.5%), and >70 years (8%) were the most common in this study. Cases in the 51–80 age range accounted for 52% of the total, which is consistent with previous research series reported by authors like Grandhi B et al [7] and Shruthi HP et al [4], respectively.

Males outnumbered females in this study (2:1), similar to previous research by Shah PY et al. and Srikoustubha et al. [8,9].

While other variables, such as occupational carcinogens including aromatic amines, polycyclic aromatic hydrocarbons contribute to the development of bladder cancer in males, smoking is the most significant. In our study also, most of the male patients with both benign and malignant lesions were chronic smokers. However, this study's finding of a higher prevalence among women may be attributable to the fact that ghutka, paan, khaini, and surti are all forms of smokeless tobacco that contain tobacco, betel nuts, saccharin, sugar-coated fennel, and heavy metals such as silver [10].

The current study included 69 non-neoplastic lesions. Among them, 53 were chronic non-specific cystitis, 7 were granulomatous cystitis, and 4 were endometriosis. Using chronic non-specific cystitis as the prevalent kind, their findings were comparable to those of studies conducted by Srikoustubha et al. [9] and Shruti HP et al [4]. Cystitis glandularis as variations of normal histology were seen in three cases. Out of 315 neoplastic lesions included in the study, 92 cases (29%) were Papillary Urothelial Neoplasm of Low Malignant Potential(PUNLMP), Papillary Urothelial Carcinoma-Low Grade were 108 cases(34%); 101 cases (32%) were high-grade; and 14 cases (5%) were muscle-invasive. This confirms the findings of a previous study by Laishram et al. [11] which indicated that 53.8% of the cases were non-invasive papillary urothelial carcinoma, 15.38% were superficially invasive bladder carcinoma, and 30.77% invaded muscle. PUNLMP is a urothelial lesion that, according to some studies, biologically poses a low risk of progression. Some of these tumors can recur. Hence, recognizing these tumors is crucial to alert clinicians for timely treatment [12].

Our examination confirmed the findings of earlier studies that the most common kind of invasive urothelial carcinoma was 77% without differentiation, similar to 86.67% and 92.13%, respectively, reported by Shruti HP et al. [4] and Goyal VK et al. [3]. Also included in this research were IUCs with clear cells and

squamous differentiation. Other cases not identified in our study were metastatic tumors from cervix and lower GI tract.

However, disease progression, response to treatment and the risk of recurrence is not dependent solely on histologic pattern and grade of the tumor. The use of supplementary methods, such as molecular tests or immunohistochemistry (IHC) will resolve most of the disputes over grading.

MIBC (Molecular Classification of Urothelial bladder Carcinoma) includes various subtypes based on comprehensive mRNA profiling and IHC markers, out of which predominant types were luminal and basal subtypes [13,14]. Molecular subtypes of MIBC may be stratified by using cost effective IHC markers which may be useful in predicting the prognosis. Various studies have found significant correlation between mRNA profiling and patterns of IHC staining as basal (CK5/6 expression) and luminal (CK20 expression) subtypes [15]. According to a meta-analysis, GATA3 and CK5/6 expression have 90% accuracy in identifying these two subtypes [16, 17].

Two primary tumour subtypes, basal-like (squamous) BASQ and luminal subtypes were identified by a consensus-based set of four markers: FOXA1, GATA3, KRT5/6, and KRT14, KRT20 [18].

KRT5, KRT 14 is expressed exclusively in basal/suprabasal layers, but absent in the luminal layers. Evidence suggests that BASQ tumours express KRT5/6 and KRT14, but lack GATA3 and FOXA1, CK20, according to studies [18,19]. The urothelium expresses FOXA1, GATA3, CK20, and uroplakin, but these proteins are only produced by tumours that develop from the urothelium. Urothelial carcinomas can be diagnosed and its prognosis can be predicted with the use of CK20 staining. A reduction in CK20 expression and positive staining for CK5/6 were seen in foci with squamous differentiation [22,23,24]. Jung et al. used 5 IHC markers CK5/6, CK20, P53, CD138 and Her2/Neu to evaluate urothelial lesions and diagnose urothelial Carcinoma in situ (CIS) and found that Her2/Neu was sensitive in diagnosing CIS [21].

Subtyping is helpful for analysing disease outcome, and several studies have shown that individuals with BASQ-type UBC had a poorer prognosis and more advanced illness at presentation [19,20].

There is a lack of accessibility to RNA expression profiling as a tool for subtype classification. Subtyping bladder cancer has therefore been shown in many investigations using different IHC panels [24,25]. Although GATA3 has been extensively examined for its predictive significance, additional IHC markers such as CK20, CK5, and CK14 have shown no clear results.

We used immunohistochemistry (IHC) with three markers—GATA3, CK5 (basal) and CK20 (luminal)—to examine the malignant cases that were available. In high grade papillary urothelial carcinoma, 75% of cases tested positive for CK5 and CK20 was negative; patients with squamous differentiation were more likely to have advanced stages and a poor prognosis. All non-invasive and invasive urothelial carcinomas showed diffuse nuclear expression of GATA3. In low grade papillary neoplasms, 80% of cases tested negative for CK5 and positive for CK20; this suggests that CK5 could be a useful prognostic biomarker for dividing NMIBC patients into high- and low-risk categories. This vibes with research by Bejarananda et al [27], Ezohery et al [25], and Al Sharaky et al. [26].

In a study to classify basal and luminal subtypes of urothelial carcinoma by IHC markers CK5, GATA3 and CK20 by Ravanini et al, it was postulated that there was no correlation between immunohistochemical subtypes and lymph node metastases, also no difference in rate of survival was observed [28]. However, there is still space for research in molecular sybtyping of urothelial

carcinomas so as to facilitate patient outcome and broaden treatment options.

Conclusion

Our study exposed that malignant bladder lesions are very common in elderly population, frequently seen in chronic smokers. Lesions in the urinary bladder may take many forms, and it is important to be aware of the many subtypes, risk factors, and histological characteristics as urinary bladder lesions are prevalent in our society. Identifying potential hazards and risks help in making a correct and prompt diagnosis. Smoking is a common factor in the development of many of these malignant tumours, which tend to affect the elderly. The prognosis and treatment considerations for invasive and non-invasive urothelial carcinomas are completely different, hence it is crucial to determine the degree of invasion by microscopic inspection. Molecular subtypes of invasive urothelial carcinoma may be identified by IHC testing. Understanding the genomic profile and IHC patterns of molecular subtypes of MIUCB(Muscle Invasive Urothelial Carcinoma of Bladder), as well as being aware of the varied histological heterogeneity of these lesions may allow us to identify cases with poor outcomes and create innovative biomarker-directed therapeutics. Subtyping tumours based on histopathology and ancillary testing like immunohistochemistry in a way that is practical for everyday clinical practice is therefore essential.

Conflict of Interest

The authors declared no conflict of interest.

Acknowledgments

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