



## **The Role p53 Protein Expression in Urothelial Neoplasm**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

The urinary bladder had a line with transitional epithelium. Urothelial neoplasms are the majority among the bladder neoplasms. Urothelial neoplasms were more common in males than in females. Urothelial neoplasms usually occur in the elderly age group. The majority of the tumor size was more significant than 3 cm, and papillary type is majorly found among the tumors. The present study aimed to identify the grade and staging of the p53 in urothelial neoplasms.

The bladder carcinoma (54) cases from Department of Pathology, Sree Balaji Medical College and hospital (during September 2015 to September 2017) were analysed. The median age for bladder carcinoma in the present study was 66 years. The clinical parameter studied was the size of the lesion based on the cytoscopic or radiological findings (31- 33). Based on tumor size, lesions were classified into two groups, less than equal to 3cm and greater than 3cm, which was found to be 42% and 58%, respectively This study concludes that p53 is useful in differentiating benign and malignant neoplasms in morphologically difficult cases. Immunohistochemistry for p53 is useful adjunct to histomorphology.

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## 1. INTRODUCTION

Bladder cancer is the seventh common cancer worldwide and the sixth most public in developed countries and comprises 3.2% of all cancers [1, 2]. It is more common in westernized countries like North America, United Kingdom and Australia. The incidence of bladder cancer in India is 3.2% in males and 0.7% in females among all malignancies. Ninety-eight percent of urinary bladder cancers are of epithelial origin, among which 90% are urothelial carcinomas [3-4]. Bladder cancer forms when the DNA in cells in the bladder change, deactivating the functions that control cell growth. The immune system criticizes these mutated cells. But some mutated cells may escape the immune system and grow out of control, forming a tumor in the bladder. Bladder cancer is mainly caused by smoking and industrial exposure [5-7]. By epidemiological studies, tobacco smoking has been occupied in 50% in men and 30% in women of bladder cancer cases [4-6]. Ninety eight percent of urinary bladder cancers are of epithelial origin, among which 90% are urothelial carcinomas. It usually presents as a non-invasive papillary tumor swelling from the surface of the mucosa. About one-third of bladder cancers presents as non-papillary, solid tumors which arise from in situ dysplasia and carcinoma in situ. These tumors attack the bladder wall and are prone for metastasis [8–11]. The 2016 WHO classification of tumors of the urothelial tract provides a review of morphology of urothelial neoplasms, emphasizing on their divergent differentiation, multiple morphologic variants and a diverse genomic landscape.

Rating is important in noninvasive disease, specifically papillary neoplasms. Noninvasive tumors divided into two categories, papillary and flat. As in 2004, the 2016 WHO classification continues to recommend the application of the grading classification first put forth by ISUP in 1997. According to the 2016 WHO classification, papillary urothelial neoplasms have been categorized as: low grade papillary urothelial carcinoma (LGPUC), high grade papillary urothelial carcinoma (HGPUC), papillary urothelial neoplasm of low malignant potential urothelial papilloma (UP), inverted urothelial papilloma (IUP), urothelial proliferation of uncertain malignant potential urothelial dysplasia (UD) [10,11-14].

Urothelial carcinoma is well known for its different variation. The most common are glandular and squamous differentiation. These replacements are major to find as it has diagnostic, prognostic and therapeutic considerations [11,15-18]. There are several prognostic factors in bladder carcinoma; histological stage and grade of the tumor have been reflected as vital factors. In some cases, tumors with greater rating and stage behave in an indolent way, whereas tumors with lower rating and stage show a high incidence of recurrence [19-23]. Hence additional prognostic information is required to guide clinicians in the management of these patients. Urothelial playing is the most directing projecting pointer in urothelial carcinoma and is a major defining parameter in the management of this disease. (TNM Scheme for bladder carcinoma), [24-26]. The important factors while assigning a T-stage include the size of the lesion and extent of invasive carcinoma. Urothelial neoplasms are generally classified into four categories according to the WHO/ ISUP 2004 consensus classification system [25,26-30].

- Hyperplasia
- Flat lesion with atypia
- Papillary neoplasms
- Invasive neoplasms

Classifying urothelial neoplasm based on histomorphology alone has certain diagnostic dilemmas because of overlap in morphological features between: -Carcinoma in situ and flat urothelial hyperplasia, -Non invasive low grade papillary urothelial carcinoma and papillary neoplasms of low malignant potential, -Non invasive low grade/ invasive high grade urothelial carcinoma and non invasive high grade urothelial carcinoma. Immunohistochemical markers along with histomorphology can be used to differentiate various urothelial neoplasms. p53 is a tumor suppressor gene that maps to the human chromosome 17p13. The mutation of which is associated with tumor progression [13]. Popov et al. studied p53 expression in 114 cases of urothelial carcinomas and found a significant correlation between histological grade and stage ( $p < 0.001$ ) [13]. Apoptosis can be induced by the binding of Caspase 9 to cytochrome c and Apaf1. P53 may activate the expression of Apaf1 and Bax. Bax can then stimulate the release of cytochrome c from mitochondria. The identification of the factor for facilitating the

prognosis is a challengeable one to analyze the risks of the patients. The present study aimed to identify the grade and staging of the p53 in urothelial neoplasms.

## 2. MATERIALS AND METHODS

54 cases were bladder cases. Among them, 2 specimens were radical cystectomies, 51 specimens were TURBT and 1 specimens were other type biopsies. The total number of non - neoplastic and malignant cases of the urinary bladder was 1 and 53 respectively.

### 2.1 Source of Data

The bladder carcinoma cases reported in the Department of Pathology, Sree Balaji Medical College and hospital from September 2015 to September 2017 and had been sent by the Department of Urology for analysis.

### 2.2 Inclusion Criteria

All the cases of urothelial neoplasms reported in bladder specimens irrespective of patient's age and sex and the procedure done were included for the study.

### 2.3 Exclusion Criteria

- Carcinomas other than urothelial carcinomas.
- Cases with inadequate material.
- Cases without deep muscle biopsy.

### 2.4 Method of Data Collection

Detailed history of the cases regarding age, sex, and history, type of procedure, site, size, stage, previous surgery details and urinary cytology were obtained for all the urothelial carcinomas reported during the study period. 4 μ thick sections of paraffin tissue specimen are stained with Hematoxylin and Eosin. Age, gender, tumour size and tumour location (base, lateral, posterior walls and trigone) were the clinical and pathological parameters evaluated. For the malignant lesion staging was done according to TNM staging for bladder carcinomas.

### 2.5 Statistical Analysis

The various histological parameters were correlated with immunohistochemical profile. The immunohistological profiles were correlated with grade and stage. Association between alteration

of marker expression and grade and stage were examined using Pearson Chi-square test. Results were considered significant if the p-value <0.05.

## 3. RESULTS

Fifty four specimen of newly diagnosed cases of bladder carcinomas were received from the department of urology during the study period. The urothelial neoplasms constituted 92.59 % of newly diagnosed bladder carcinomas, whereas the non urothelial neoplasms were 7.40%.

The distribution of bladder carcinomas is shown in Table 1

**Table 1. Distribution of bladder neoplasm**

	No. of cases	Percentage
Urothelial Neoplasm	50	92.59%
Non Urothelial	4	7.40%
Total	54	100%

Among the non-urothelial neoplasms squamous cell carcinoma were 75% and adenocarcinoma were 25%.

### 3.1 Sex Distribution

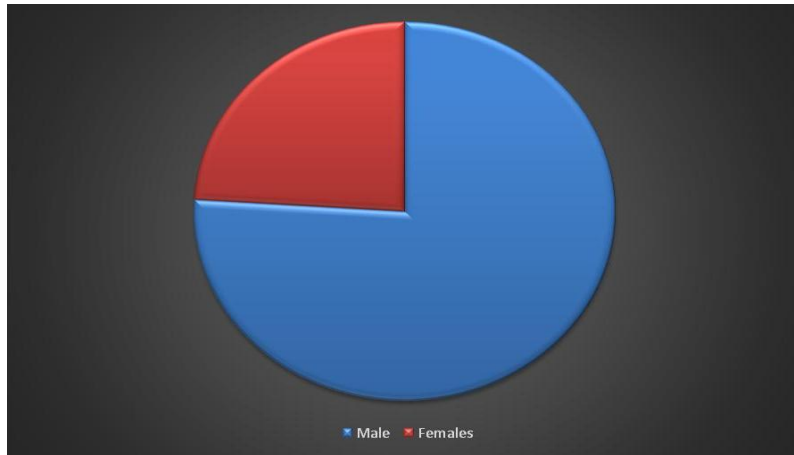
The incidence of urothelial neoplasm in males accounted for 83.01% whereas in females it was about 16.98 % and the ratio of male: female ratio 3.1:1 (Fig. 1).

### 3.2 Age Distribution in Urothelial Neoplasm

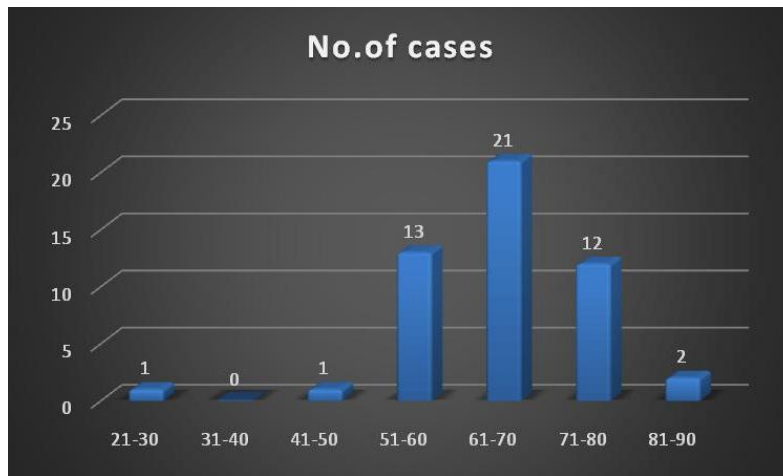
The median age for urothelial neoplasm in our study group was 66 years. The age of patients ranged from 24 to 82 years. Majority of the cases were between the 5<sup>th</sup> to 8th decade (Fig. 2).

### 3.3 Size of the Tumor

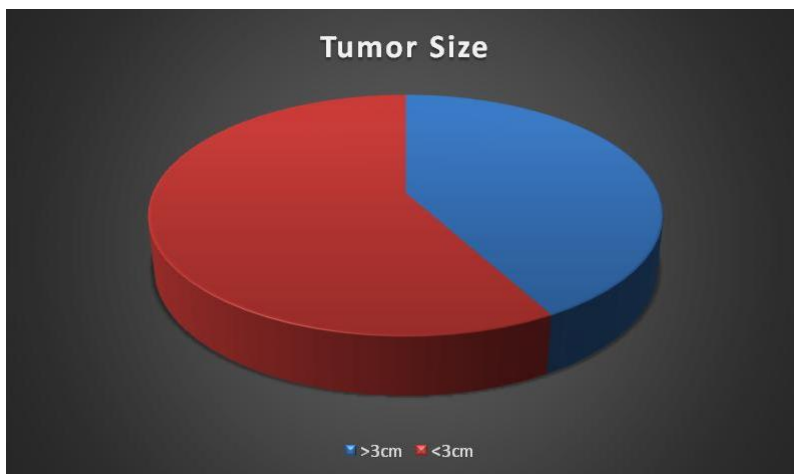
The size of the lesions was noted from the cystoscopic/ radiological findings, the tumor size was the largest of that noted in the three dimensions, and the largest of that is taken as tumor size. The largest tumor size was considered in case of multiple lesions. The tumors were grouped into two based on the size (greater than 3 and less than or equal to 3). In our study majority of the cases, 58 % were greater than 3cm (Fig. 3).



**Fig. 1. Sex distribution in urothelial neoplasms**



**Fig. 2. Age distribution in urothelial neoplasms**



**Fig. 3. Tumor Size**

### 3.4 Tumor Location

Based on the cystoscopic and radiological findings majority (48%) of the tumors were located in the lateral walls (anterolateral, posterolateral, right lateral and left lateral ), 22% was in the anterior and 18% in posterior walls, although less in frequency few were seen in bladder neck and trigone (Fig. 4).

### 3.5 Papillary Neoplasm

All the urothelial neoplasms were papillary in this study which includes only one benign and the rest were malignant neoplasms.

Papilloma: (Fig. 3. A & B)

PUNLMP: (Fig. 4. A, B & C)

Low grade: (Fig. 5. A, B, C, D & E)

High grade: (Fig. 6. A, B, C, D, E & F)

### 3.6 Histomorphological Analysis

Certain histomorphological features of the urothelial neoplasms were studied and correlated with tumor grades in this study. Histomorphological features studied were presence of umbrella cell, prominent nucleoli and number of mitosis (absent, <5, 5-10, >10). Prominent nucleoli and number of mitosis were significant in differentiating between the benign and malignant lesions. High grade neoplasms have cohesive papillae and varying degrees of

nuclear pleomorphisms and prominent nucleoli. In classical cases, differentiating between low and high grade carcinoma is not difficult with certain limitations. Preserved umbrella cell layer is considered to be a feature of low grade carcinomas, but in this study, around 40% of high grade carcinomas had preserved umbrella cell layer. A number of mitosis were significant in differentiating low grade and high grade carcinomas. Low grade papillary carcinomas had mitosis less than 5/10 hpf, whereas in high grade carcinomas mitosis were 0 - 30 /10 hpf. High grade carcinomas had 1 -2 prominent nucleoli (Tables 2 and 3).

### 3.7 Immunohistochemistry

In the present study, immunohistochemistry was performed in 27 cases diagnosed with urothelial neoplasm and p53 marker expression was studied.

### 3.8 p53 in Urothelial Neoplasms

In this study, the benign case i. e., papilloma was negative (<10%) for p53. Twenty five (25%) percent of PUNLMP was positive (>10%) for p53 and the percentage of positivity was around 20% which is considered as grade 1. (8/10) 80% of low grade carcinoma and (11/ 12) 91.6 % of high grade carcinomas showed >10% p53 expression. p 53 expression was graded into 5 grades (grade 0, 1, 2 ,3,4).

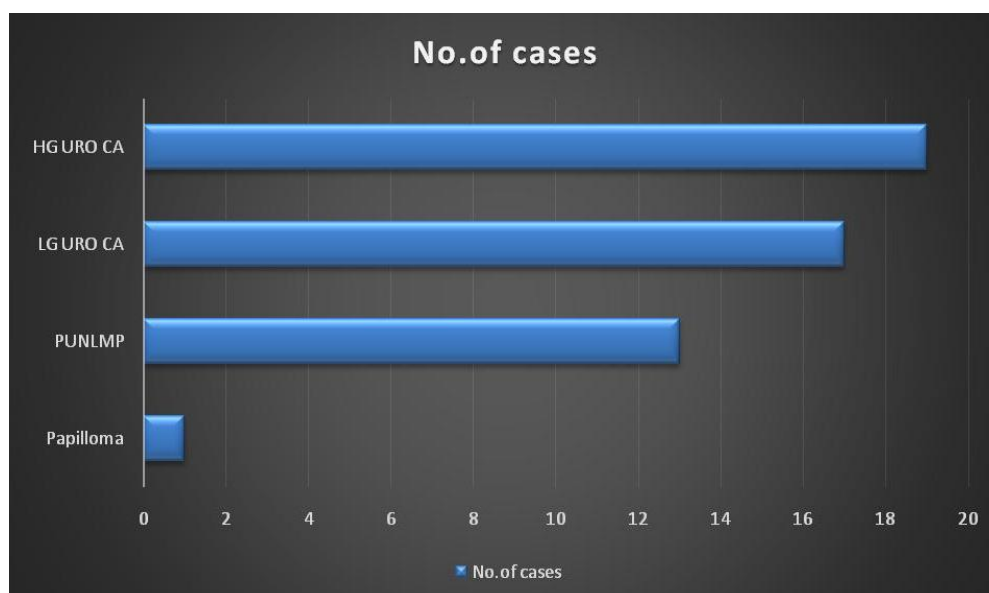


Fig. 4. Papillary lesions of urothelium

**Table 2. Histomorphological features**

Tumor Grade	Number of mitosis					P-Value	P	Prominent nucleoli			P	Umbrella cells		
	A	< 5	5 - 10	> 10	Total			A	Total	P-Value		A	Total	P-Value
Benign	1	0	0	0	1		0	1	1		1	0	1	
With Malignant Potential	12	1	0	0	13		1	12	13		8	5	13	
Malignant	2	15	6	13	36	0.000	14	22	36	0.001	17	19	36	0.045
Total	15	16	6	13	50		15	35	50		26	24	50	

*A- Absent, Malignant Potential- lesions with malignant potential.  
P-value is significant between benign, with Malignant Potential, for number of mitosis and prominent nucleoli.*

**Table 3. Histomorphological difference between low grade and high grade carcinomas**

Tumor grade	Number of mitosis					P-Value	P	Prominent nucleoli			P	Umbrella cells		
	A	< 5	5 - 10	> 10	Total			A	Total	P-Value		A	Total	P-Value
Low Grade	1	14	1	1	17		12	5	17		3	14	17	
High Grade	1	2	7	9	19	0.000	11	8	19	0.42	6	13	19	0.33
Total	2	16	8	10	36		23	13	36		16	20	36	

*A- Absent,  
p-value is significant between low grade and high grade carcinomas for number of mitosis.*

### 3.9 Expression of p 53 in Low Grade and High - Grade Carcinomas

91.6 % of high grade carcinomas showed >10% p53 expression. Percentage of cells expressing p53 was not different between the two groups. The number of cases was distributed randomly between the grades of p53. p-value was not significant (p=0.2). The intensity of p53 expression and level of p53 positivity will help in differentiating the two grades. Diffuse and very intense positivity was noted in high grade carcinomas. The difference in p53 expression was not significant between low grade and high grade carcinomas (Table 4).

### 3.10 Staging in Urothelial Neoplasm

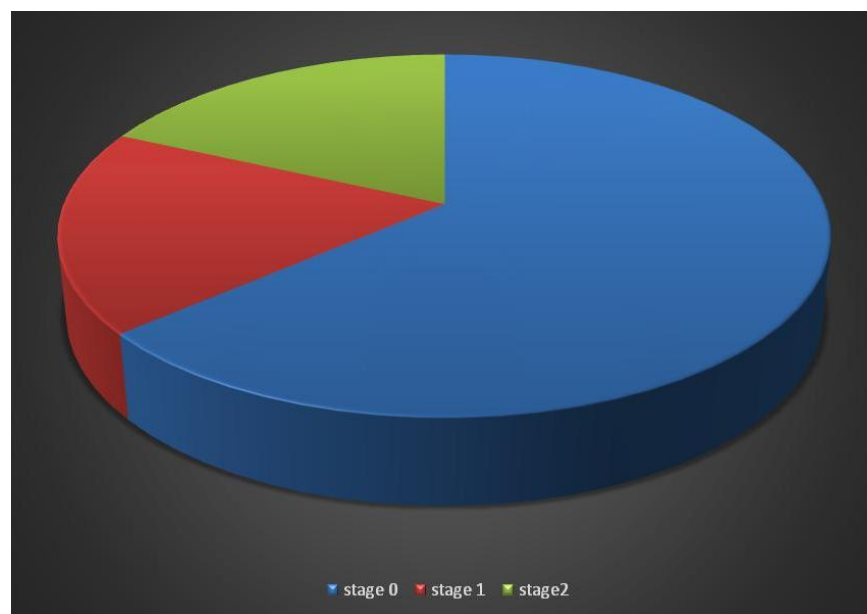
Staging was performed in all cases of TURBT specimens which included low grade papillary

non-invasive, high grade papillary non invasive, high grade invasive.(10+4+8). Sixty three percent (63%) were non-invasive and 36.36% were invasive carcinomas (Fig. 5).

p53 expression >10 % was seen in 78.57% of non-invasive urothelial carcinomas (stage p Ta), 100 % urothelial carcinomas with lamina propria invasion (stage pT1) and 75 % of the muscle-invasive urothelial carcinomas (pT2). All the stages of carcinomas showed variable distribution between the variable grades of tumor. The difference in p53 expression between the stage of tumor was not statistically significant (p>0. 05). Difference in p53 expression between stages of carcinoma was not significant (Table 5 and Figs. 6 - 15).

**Table 4. Expression of p53 in low grade and high grade carcinoma**

	P53					Total	P-Value
	0 (010%)	1 (11-25%)	2 (2650%)	3 (51-75%)	4 (> 75%)		
Low grade pap carcinoma	2	1	2	4	1	10(100%)	
High grade pap carcinoma	1	1	1	2	7	12 (100%)	0.2
Total	3	2	3	6	8	22	

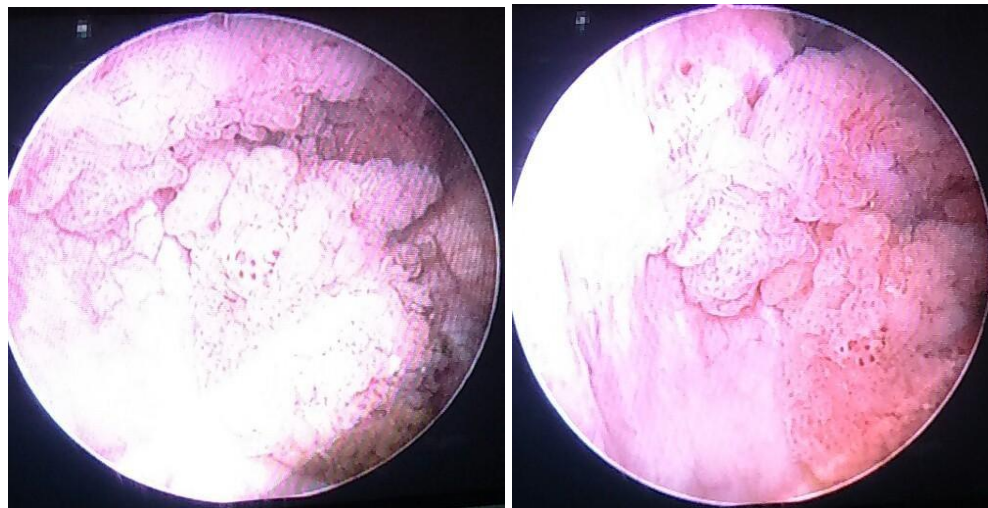


**Fig. 5. Distribution of urothelial carcinomas in various stages**

**Table 5. p53 expression and stage of urothelial carcinoma**

	<b>P53</b>					<b>Total</b>	<b>P-Value</b>
	<b>0 (0-10%)</b>	<b>1 (11 -25%)</b>	<b>2 (26 -50%)</b>	<b>3 (51 -75%)</b>	<b>4 (&gt; 75%)</b>		
<b>Non Invasive(pTa)</b>	3(21.4%)	1(7.14%)	3(21.4%)	5(35.71%)	2(14.2%)	14( 100%)	
<b>Invasive(pT1 )</b>	0	0	1(25%)	1(25%)	2(50%)	4(100%)	
<b>2(pT2b)</b>	1(25%)	0	1(25%)	0	2(50%)	4(100%)	
<b>Total</b>	4	1	5	6	6	22	0.719

**MICROGRAPHS**



**Fig. 6. A&B. Cystoscopy showing papillary fronds**





Fig. 7. CT scan showing bladder growth

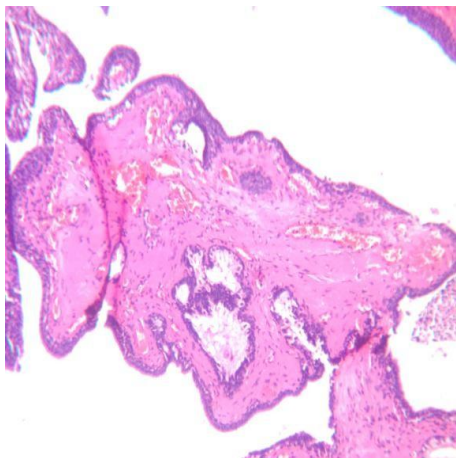


Fig. 8A. H &E: A. Papilloma (100x mag.)

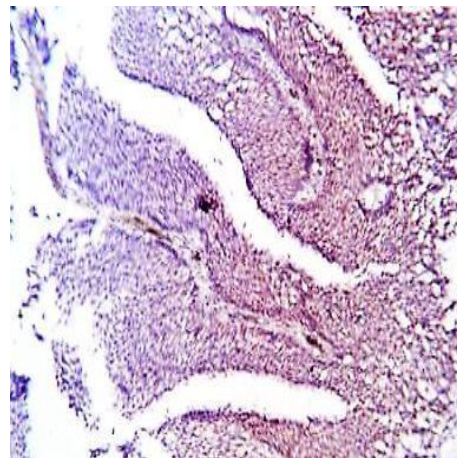


Fig. 8B. p53 expression in Papilloma (100x mag) Grade 0

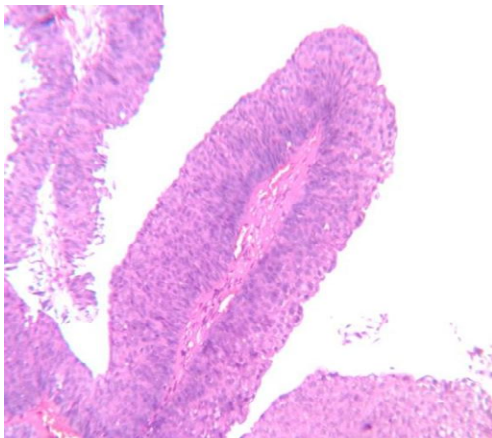


Fig. 9A. H &E: PUNLMP (100x mag.)

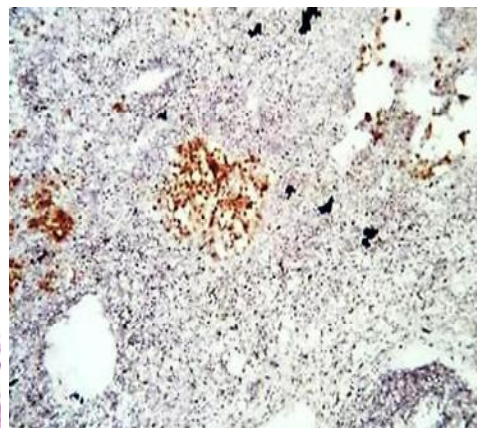
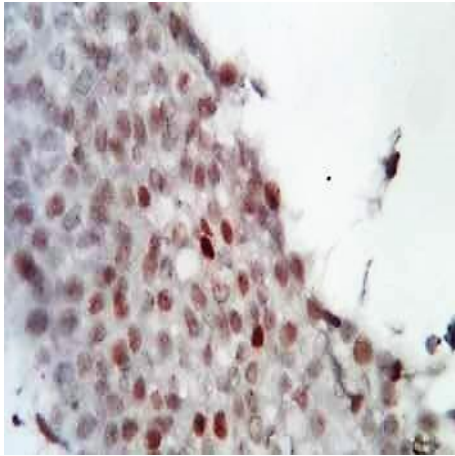
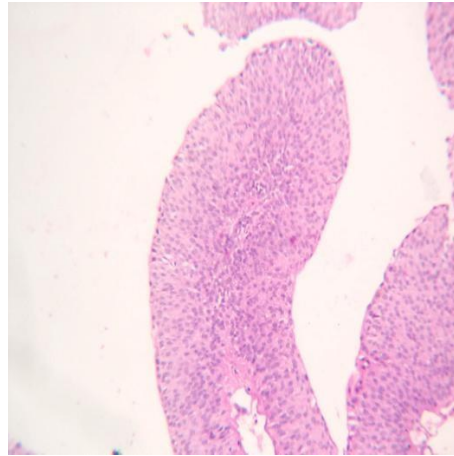


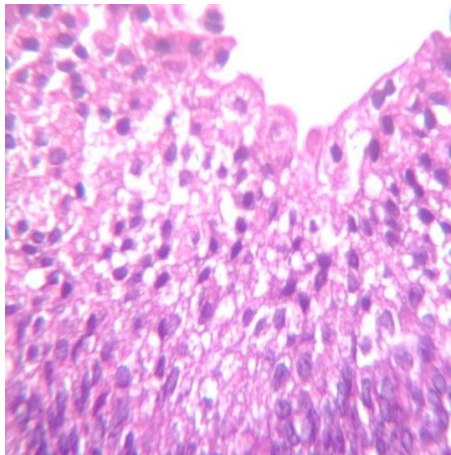
Fig. 9B. p53 expression in PUNLMP(100x mag.) Grade 1



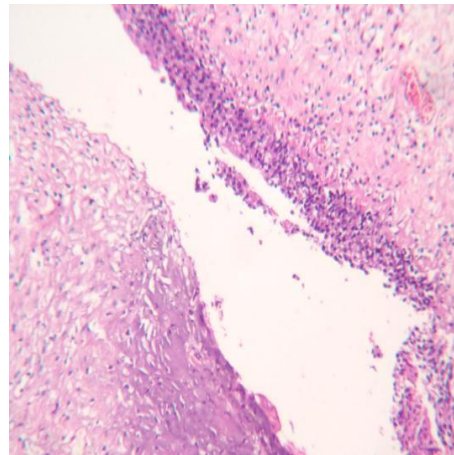
**Fig. 10A. p53 expression in PUNLMP(400x mag.) Grade 1**



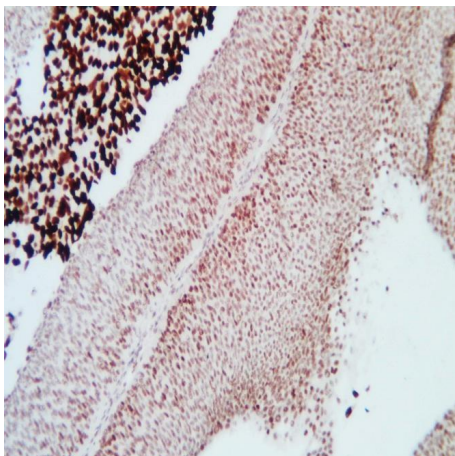
**Fig. 10B. H&E: Low grade urothelial carcinoma**



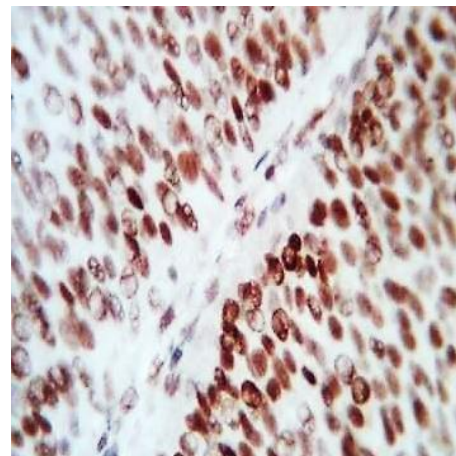
**Fig. 11A. Showing umbrella cell layer**



**Fig. 11B. No Deep muscle invasion**

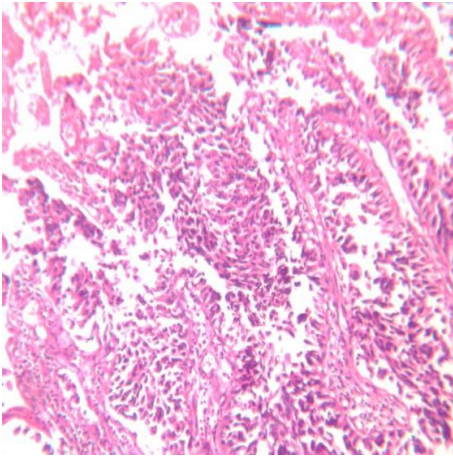


**Fig. 12A. p53 expression in low grade (400x mag.)**

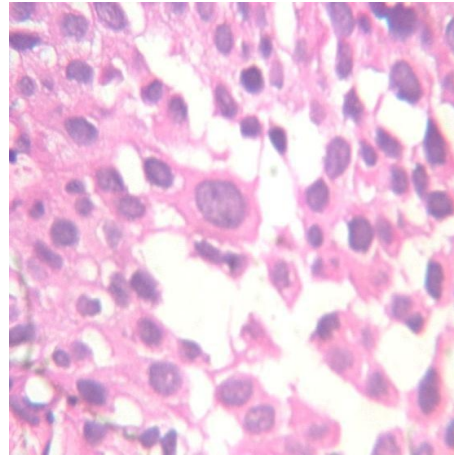


**Fig. 12B. p53 expression in low grade (100x mag.) Grade 3**

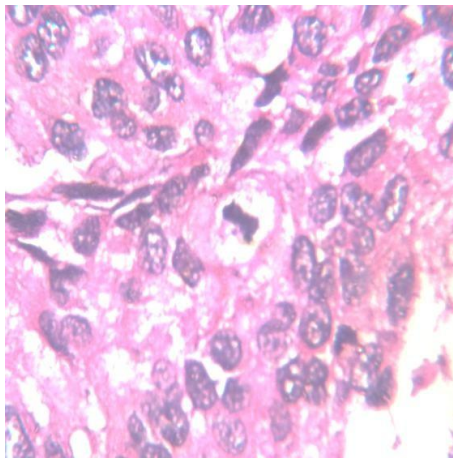




**Fig. 13A. H&E. High grade carcinoma (100x mag)**



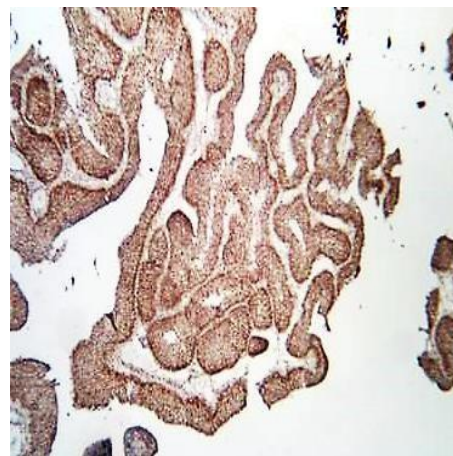
**Fig. 13B. Showing nuclear pleomorphism**



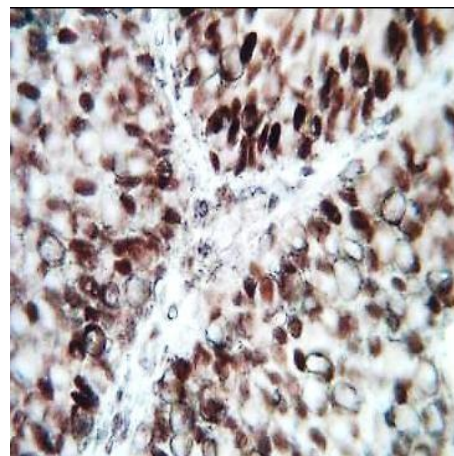
**Fig. 14A. H&E. Showing Mitosis (100x mag.)**



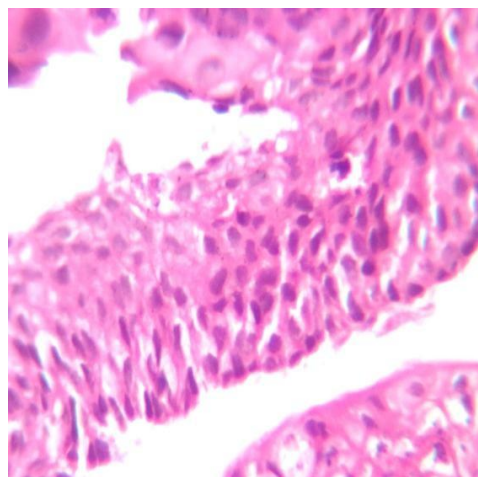
**Fig. 14B. p53 expression in high grade(100x mag.) Grade 4**



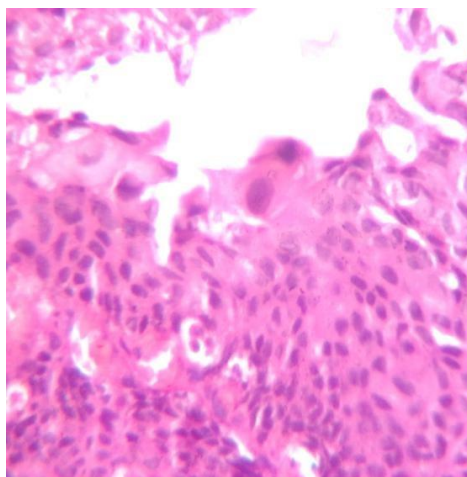
**Fig. 14C. p53 expression in high grade urothelial carcinoma(100x mag.)**



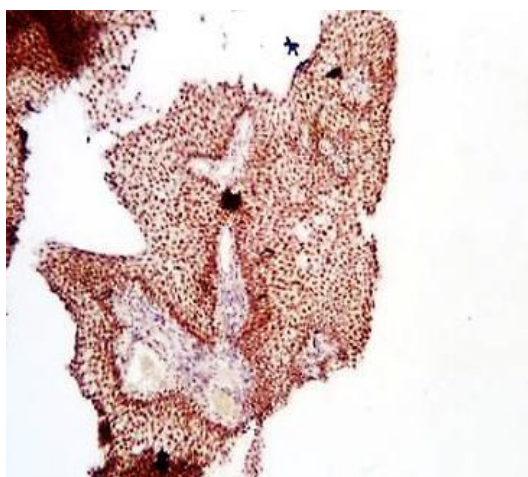
**Fig. 14D. High grade urothelial carcinoma(400x)**



**Fig. 15A. H&E A. High grade urothelial neoplasm with squamous differentiation (100x mag.)**



**Fig. 15B. H&E B. High grade urothelial neoplasm with squamous differentiation (400x mag.)**



**Fig. 15C. p53 expression in high grade urothelial neoplasm with squamous differentiation (100x mag.) Grade 4**

#### 4. DISCUSSION

During the study period of 2 years from September 2015 to September 2017, 54 new cases of bladder neoplasms were diagnosed. Out of which one was papilloma. Fifty three (53) cases were of urinary bladder carcinoma. The most common type was urothelial carcinoma accounting for 92.45%, followed by squamous cell carcinoma and adenocarcinoma accounting for 5.66% and 1.88%, respectively. Khare V et al. [23] and Sadetzki et al. [28] reported the incidence of transitional cell carcinoma to be about 90% in their respective studies. Urothelial cancers were more common in males than

females, with male to female ratio being 3.1:1. This was in concordance with previous studies by Melissa et al. [20] and C. Aparna et al. [25], which found the sex ratios to be 1.8:1, 5:1 respectively. In the present study, 92% of cases occurred in the 5<sup>th</sup> to 8<sup>th</sup> decades. Age range was found to be between 24-82 years. Aparna et al. [25] found around 75% of cases in 4<sup>th</sup> to 7<sup>th</sup> decade. In the present study, 77.77% were males and 22.22% were females which were below 70 years of age. In the study conducted by Aparna et al. [25] among the patients below 70 years, 72.22% were males and 27.77% were females.

The present study was found to be concordant with the previous study done by Chang et al. [26]. In the present study, urothelial neoplasms were classified according to WHO/ ISUP 2016 classification. The majority of the lesions were papillary, this was similar to the findings of Enache et al. where the frequency of papillary carcinoma was 95.5%. The noninvasive cases were 72.22% and 27.77% were invasive urothelial carcinoma. Kalantari et al. [28], found 60% of the cases non invasive and 40% of cases invasive urothelial carcinoma. According to them, the Non invasive papillary carcinoma, 66.66% were low grade papillary urothelial carcinoma, 33.33% were PUNLMP.

Bahadir et al. [25], found 83.80% of non-invasive was low grade papillary carcinoma and 18.80% was high grade carcinoma. According to Bahadir et al., 15.1% of cases were stage p T1 and 5.1% in stage p T2. Sen et al. [30], found 27% of the cases to be p T1 and 4.2% of the cases to be pT2. The incidence of stage p T1 tumor was 7.4% and the incidence of muscle-invasive stage p T2 was also found to be 7.4% in the present study. This was accordance with the previous study [31-34].

In the present study, p53 expression of <10% and >10% was taken into consideration. Kalantari et al. [28] found 25% of low grade carcinomas with <10% p53 expression. In our study, cases with <10% p53 expression, which is grade 0 was seen in 20% of the low grade carcinomas. According to the same author, 85% of the high grade carcinomas showed >10% p53 expression, which was concordant with our study which revealed 91.66% of the high grade carcinomas with >10% p53 expression (Grade 1,2,3, 4). Sun et al. [30] studied p53 expression and found 21.4% of low grade carcinomas showed <10% expression and 100% of high grade urothelial carcinoma s showed >10% p53 expression. P-value was not significant with tumor grade and p53 in both the studies (>0.05). p53 and stage of the tumor S. DiCioccio et al. [35], compared p53 expression with the stage of urothelial carcinomas. The author found that 20% of pTa, 20.28% of p T1 cases and 19.35% of pT2 cases with <10% p53 expression. In the present study 21.4% of p Ta cases, none of the pT1 cases and 25% of pT2 cases showed <10% p53 expression. According to the same author, 80% of the p Ta cases, 79.71% of p T1 cases and 80.64% of pT2 cases showed >10% p53 expression. In the present study 78.57% of pTa cases, 100% of pT1 cases and 75% of pT2

cases revealed >10% of p53 expression. However, p-value was not significant.

Koyuncuer et al. [32], found a statistically highly significant relationship between pT1 and p T2 regarding p53 staining percentages (P < 0.01); the staining percentage of pT1 below 10% and the staining percentage of p T2 by 10% or over were determined significantly high. Whereas 17 (60.7% of all p T1 cases) of 20 pT1 and pT2 cases that showed p53 staining percentage below 10% were p T1, 13 (81.3% of all pT2 cases) of 24 p T1 and pT2 cases that showed immunoreactivity over 10% were pT2. In the present study, all p T1 stage carcinomas showed >10% p53 expression and 75% of pT2 stage carcinoma showed >10% p53 expression. This difference in p53 expression was not significant according to the present study. According to the present study, p53 expression will be useful in differentiating nonmalignant from malignant cases.

Kalantari et al. [28], studied p53 expression with tumor grades and stage of urothelial carcinomas. The author found that all PUNLMP cases were negative for p53 and the difference between in p53 expression was statistically significant between different types (PUNLMP, low grade, high grade carcinomas). In this present study, 3 out of 4 cases showed negativity for p53, 1 case showed grade 1 positivity for p53 and none of the cases showed >25% positivity for p53 in the PUNLMP category.

## 5. CONCLUSION

Morphology plays a major role in the differential diagnosis of urothelial neoplasms. Negative p53 staining in PUNLMPs and high p53 index in high grade papillary urothelial carcinomas and invasive carcinomas supported the involvement of p53 mutation in the development of urothelial neoplasms but plays a crucial role in progression of the malignancy.

## CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

## ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.



## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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