

## Utility of Cell Block with p53 Immunostain in Diagnosing Urothelial Carcinoma

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### ABSTRACT

**Background:** Urine cytology is an initial noninvasive screening test done in patients with suspected urothelial carcinoma. The objective of this study was to compare the sensitivity of conventional smear cytology with cell block technique for the diagnosis of malignant cells in urine and to assess the efficacy of p53 immuno-marker in the diagnosis and prognosis of urinary bladder cancer.

**Methodology:** The study was carried out in the department of Histopathology, Pakistan Institute of Medical Sciences(PIMS), Islamabad. Cell blocks and cytology smears were prepared from urine samples of 80 patients of urothelial carcinoma and p53 stain was applied. Data was analyzed using SPSS version 21. Sensitivity of conventional smear and cell block techniques were calculated for both low and high grade urothelial carcinomas.

**Results:**The sensitivity of detecting atypical cells using conventional smear was 31% and 70.6% for low grade and high grade urothelial tumors respectively. The sensitivity increased to 41.4% and 84.3% for low and high grade urothelial carcinomas respectively with cell block technique. Positive p53 stain was seen in 13.8% of cases with low grade tumors and 66.7% cases with high grade tumors. Chi-square test was used to find the association of cytology findings with the histologic grade.

**Conclusion:** Sensitivity of urine cell blocks is higher than the conventional smear method for detecting high grade lesions. It can be used as an efficient non-invasive technique and an adjunct tool for the determination of disease relapse and overall survival in patients.

**Keywords:** Carcinoma, Cytology, P53 Protein, Urine

#### Authors' Contribution:

<sup>1,2</sup>Conception; Literature research; manuscript design and drafting; <sup>3,4</sup> Critical analysis and manuscript review; <sup>5,6</sup>Data analysis; Manuscript Editing.

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### Introduction

Carcinoma of urinary bladder is the 9th most common malignancy worldwide.<sup>1</sup> Risk factors include, male sex, aging, tobacco, Schistosoma

haematobium infection, occupational carcinogens and genetic alterations, p53 being the most important.<sup>2</sup>

Urine cytology is performed as an initial evaluation routine test for suspected bladder carcinoma although cystoscopic examination and biopsy are currently the gold standard method.<sup>3</sup> It has a low sensitivity for detecting urothelial carcinoma but still performed as it is non-invasive and relatively inexpensive.<sup>4</sup> In patients with bladder cancer, the diagnostic accuracy is enhanced if cell blocks are made together with the conventional smears.<sup>5</sup> Cell blocks are also useful for special stains and immunohistochemical staining.<sup>6</sup>

This study aims to determine whether urine cell block technique can improve the diagnostic sensitivity as compared to urine cytology alone for detecting urothelial carcinoma and also to evaluate the importance of p53 mutations and its diagnostic and prognostic value in bladder cancer. Some of the previous international studies strongly support this view while according to some authors, cell block technique has no significant role in improving the diagnostic utility of urine cell cytology.<sup>7,8</sup> We didn't find any local study done regarding cell block technique in urine cytology.

P53 expression is the most common gene involved in human cancers. P53 positivity on urine cell blocks can be used as a useful marker for assessment of urinary bladder cancer prognosis as P53 mutation is associated with increased recurrence rate of bladder cancer.<sup>9</sup> We also added p53 immunohistochemistry for even better results and to check the possibility of using cell blocks for ancillary techniques like immunohistochemistry.

## Methodology

This cross-sectional study was conducted in the department of Histopathology, Pakistan Institute of Medical Sciences (PIMS), Islamabad after approval from ethical committee at Shaheed Zulfiqar Ali Bhutto Medical University. The study duration was 4 years (2016-2019). Sample size was calculated to be 80 by using WHO sample size calculator taking 95%

confidence level and 60% anticipated population proportion.<sup>10</sup>

Consecutive samples of 80 patients with diagnosis of urothelial carcinoma on histopathology were included in the study after taking informed consent from the patients. Patients with other urinary bladder tumors, PUNLUMP and other reactive conditions and those with inadequate smears were excluded. Smears with any atypical cells were considered satisfactory as adequacy criteria for urine cytology and blocks is not yet established so those smears which didn't show any atypical urothelial cells were considered inadequate were excluded.<sup>11</sup>

About 30 ml freshly voided samples of urine were collected from these patients, centrifuged immediately at 3000 revolutions per minute for ten minutes and subjected to the conventional smear cytology and cell block technique. From each of the case, two urine cytology smears were selected and each of the smear contained at least 10 atypical cells. These two slides were subjected to Hematoxylin and Eosin (H&E) staining. Immunohistochemical staining for P53 was applied on sections made from all cell blocks. Cell block containing at least 5% positively stained nuclei were labelled as positive P53 staining.<sup>12</sup>

Data was analyzed using SPSS software version 21. Sensitivity of conventional smear and cell block techniques were calculated for both high and low grades of urothelial carcinoma. Specificity calculation was not useful in this study since already diagnosed cases of urothelial cancer were included and no true negatives or false positives were present in this study. Chi-square test was applied to calculate the relationship between P53 expression and tumor grade. p values were also calculated to evaluate the statistical significance of conventional smear and cell block technique for low and high grades of urothelial cancer. p value of  $\leq 0.05$  was taken as significant.

## Results

Among 80 study cases, 18 (22.5%) were females and 62 (77.5%) males. Male to female ratio was 3.4:1.

The peak age was 50-70 years for both males and females (range: 21-87 years) (Table 1).

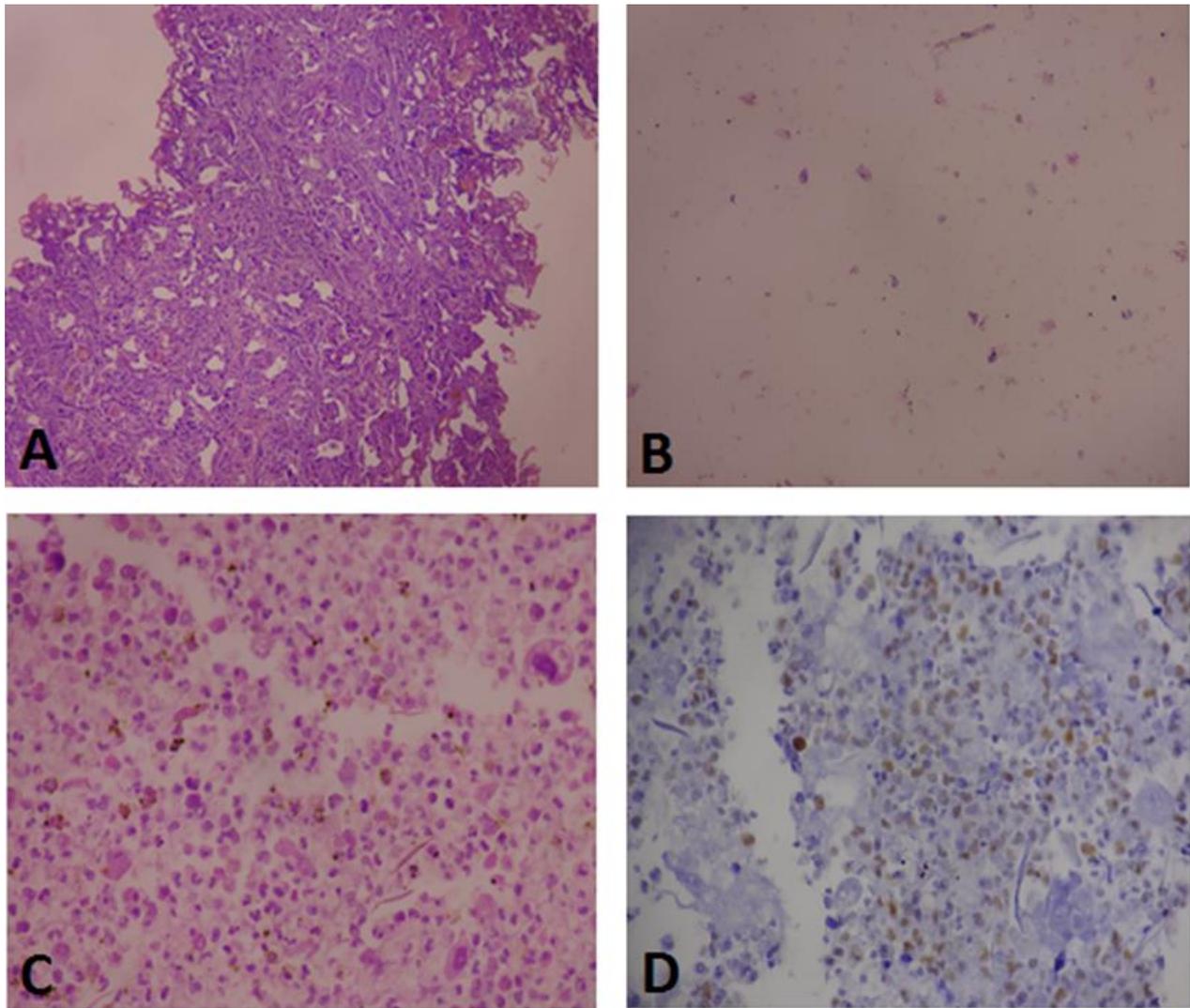
| Age Range (years)     | 21-30   | 31-40   | 41-50     | 51-60     | 61-70     | 71-80     | 81-90    |
|-----------------------|---------|---------|-----------|-----------|-----------|-----------|----------|
| <b>Males (n=62)</b>   | 1(1.6%) | 4(6.4%) | 8 (12.9%) | 17(27.4%) | 22(35.4%) | 8(12.9%)  | 2(3.2%)  |
| <b>Females (n=18)</b> | 0 (0%)  | 0 (0%)  | 1 (5.5%)  | 7 (38.8%) | 4 (22.2%) | 6 (33.3%) | 0 (0%)   |
| <b>Total (n=80)</b>   | 1(1.2%) | 4(5%)   | 9(11.2%)  | 24(30%)   | 26(32.5%) | 14(17.5%) | 2 (2.5%) |

Among 80 cases of urothelial cancer, 37% cases (29/80) were low grade while 63% cases (51/80) were diagnosed as high grade urothelial carcinoma on surgical biopsy.

With conventional smear method, atypical cells were detected in 31% low grade & 70.6% high grade urothelial carcinoma cases. With cell block technique, atypical cells were seen in 41.3% cases of

low-grade & 84.3% cases of high grade urothelial cancer. Atypical cells were identified in 10 additional cases by cell block method. Malignant cells were missed in 35 (43.8%) cases by conventional smear and 25 (31.2%) cases by the cell block method. (Table 2).

|                           | Conventional Smear           |                              | Cell Block                   |                              | P53 staining |            | P Values                                   |
|---------------------------|------------------------------|------------------------------|------------------------------|------------------------------|--------------|------------|--|
|                           | Positive for malignant cells | Negative for malignant cells | Positive for malignant cells | Negative for malignant cells | Positive     | Negative   |  |
| <b>LGUC (n=29)</b>        | 9 (31%)                      | 20 (69%)                     | 12 (41.4%)                   | 17 (58.6%)                   | 6 (20.7%)    | 23 (79.3%) | 0.000                                      |
| <b>HGUC (n=51)</b>        | 36 (70.6%)                   | 15 (29.4%)                   | 43 (84.3%)                   | 8 (15.7%)                    | 34 (66.7%)   | 17 (33.3%) | 0.000                                      |
| <b>Total cases (n=80)</b> | 45 (56.2%)                   | 35 (43.8%)                   | 55 (68.8%)                   | 25 (31.2%)                   | 40 (50%)     | 40 (50%)   | (p53 staining among grades of tumor) 0.006 |



**Figure 1: Microscopic Examination of a High-Grade Urothelial Carcinoma.**

Figure 1; High grade urothelial carcinoma A, Biopsy (H&Ex400). B, Cytology smear showing atypical urothelial cells (H&Ex400).C, Cell block showing few atypical cells (H&Ex400). D, P53 immunostaining positive (x400).

Sensitivity for low grade urothelial carcinoma by conventional smear method was 31%.

Sensitivity for high grade urothelial carcinoma by conventional smear method was 70.6%. ( 36 out of 51 cases)

Sensitivity for low grade urothelial carcinoma by cell block method was 41.4%. (12 out of 29 cases)

Sensitivity for high grade urothelial carcinoma by cell block method was 84.3%. ( 43 out of 51 cases)

With the use of cell block technique, the sensitivity improved.

Chi-square test was employed to find out the significance of tumor grade with cytology results considering p values of 0.05 or less to be statistically significant. In our results p values were found to be significant for both low grade (p=0.000) and high grade (p=0.000) urothelial cancers.

P53 positivity was seen more in the cases of high grade urothelial cancer (Table 2)

P value was calculated to determine the significance of association of p53 immune stain expression with different histopathological grades of urothelial carcinoma. Significant association was observed between P53 expression for high grade urothelial cancer (p=0.006) urothelial cancers. (Table 2)

## Discussion

Routine cystoscopies are done at regular intervals for follow up of patients, it costs high and about 10% of the lesions are overlooked.<sup>13</sup> There is obviously a need for a non-invasive method for timely detection of urothelial cancer, in order to lessen the number of cystoscopies. Conventional smear and cell block methods save the patient from undergoing repeated invasive procedures like cystoscopic biopsy. In a developing country like Pakistan, it can save money and hospital resources which can be utilized effectively where needed.

Cytology is a widely used non-invasive test though its use is restricted by its poor sensitivity extending from 28-100% (median: 48%) reported by different authors. It is useful in diagnosing high grade tumors, however it is less sensitivity for low-grade urothelial tumors.<sup>15</sup>

The current study demonstrates that urine cytology and cell block methods are useful adjuvant tests in establishing the diagnosis of urothelial cancer. Cell block method is cheap, simple to perform and help in better preservation of cells in comparison to conventional smear method.<sup>16</sup> It also reduces the gap between cytology and histology. Paraffin embedded cell blocks can be handled like a biopsy specimen and multiple sections are available for other ancillary studies.<sup>17</sup>

The sensitivity using conventional smear was 31% and 70.6% for low grade and high-grade tumors respectively. The sensitivity rates of urine cytology reported by Yafi et al were 18.3% for low grade and 51.3% for high grade urothelial cancer.<sup>18</sup>

Santwani et al reported that cell block technique demonstrates better architectural patterns,

increases the positive results and can be used for application of different special stains and immunohistochemical markers.<sup>19</sup>Qamar et al evaluated the association of immunohistochemical expression of P53 with grade and stage of urothelial cancers and found that only 16% cases of low grade carcinoma showed P53 positivity whereas 91% cases of high grade carcinoma were P53 positive.<sup>20</sup> In our study, cell blocks from low grade urothelial cancer showed 20.7% P53 positivity and from high grade urothelial cancer showed 66.7% P53 positivity. Mumtaz et al found that P53 positive expression was seen in 72.9% cases of high grade urothelial carcinoma and only 36.2% cases of low grade tumors.<sup>21</sup> According to V Nassai et al ,39% cases of high grade urothelial carcinoma while 11% of low grade urothelial carcinoma were positive for p53 immunostain.<sup>22</sup> Thakur et al found p53 expression more in the high grade urothelial carcinomas (52 cases) as compared to 32 cases of low grade.<sup>23</sup> This study demonstrates a sensitivity and specificity of cytologic diagnosis comparable to previously reported studies. Cell block technique is clearly superior to conventional smears for diagnosis of atypical urothelial cells in urine. The results of both conventional smears and cell blocks were similar to the results found in literature. Urinary cytology and cell block techniques can serve as non-invasive adjuncts to biopsy for detecting high-grade lesions. Their usefulness is limited to low-grade tumors. The major limitation of this study is selection of those cases already diagnosed with urothelial carcinoma. Including those as well would have strengthened the study. Another limitation is low cellularity in urine samples and some conditions like infections, calculi or presence of blood may have masked atypical cells in the urine sample thus affecting the results.

## Conclusion

Cell block technique is superior to conventional smears for diagnosis of atypical urothelial cells in urine. Urinary cytology and cell block techniques can serve

as non-invasive adjuncts to biopsy for detecting high-grade lesions. Their usefulness is limited to low-grade tumors. Additionally, cell blocks can also be used for ancillary studies like immunohistochemistry.

## Recommendations

Comparison of immunostaining on biopsy with that on cell blocks should be done.

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