

Original Article

Immunoexpression of p53 and Ki-67 in Urinary Bladder Carcinoma and Their Association with Tumor Grade and Muscle-invasiveness

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Abstract:

Background: Urinary bladder carcinoma is one of the threatening causes of human morbidity and mortality. Various molecular studies and prognostic biomarkers are helpful to assess the outcome of urinary bladder carcinoma. High expression of p53 and Ki-67 represents the aggressiveness of the disease and indicates poor prognosis.

Methods: This cross-sectional study was conducted at the Department of Pathology, Sylhet MAG Osmani Medical College from September 2018 to July 2020 to observe the immunoexpression of p53 and Ki-67 in urinary bladder carcinoma and their association with tumor grade and muscle-invasiveness. Total 57 transurethral resection (TURBT) samples of radiologically and clinically suspected bladder tumor cases were diagnosed histopathologically included in the study.

Results: Age of the study patients ranged from 35 to 80 years with a mean of 60.60 ± 11.14 and male to female ratio was 4.7:1. There were 31 (54.4%) high grade and 26 (45.6%) low grade urinary bladder carcinomas on histopathological examination. Thirty (52.6%) cases were histopathologically muscle invasive bladder carcinoma (MIBC) and 27 (47.4%) cases were non-muscle invasive bladder carcinoma (NMIBC). Positive p53 expression was observed in 24 (77.4%) cases of high grade carcinoma while only in 6 (23.1%) cases of low grade urinary bladder carcinoma. Positive p53 expression was seen in 22 (73.3%) cases of MIBC and 8 (29.6%) cases of NMIBC. Ki-67 expression was found positive in 27 (87.1%) cases of high grade carcinoma while only in 7 (26.9%) cases of low grade urinary bladder carcinoma. Positive Ki-67 expression was seen in 25 (83.3%) cases of MIBC and 9 (33.3%) cases of NMIBC. Statistically significant association ($P < 0.05$) was observed in histopathological grades and muscle invasiveness of UBC with p53 and Ki-67 expression.

Conclusion: p53 and Ki-67 immunomarker may be helpful to provide additional prognostic information in urinary bladder carcinoma.

Key words: Urinary bladder carcinoma, MIBC, NMIBC, p53, Ki-67, immunoexpression

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Introduction:

Urinary bladder cancer is one of the most frequent malignant tumors in the urinary system¹.

It is the 7th most common cancer in men and the 17th in women².

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Worldwide newly diagnosed urinary bladder carcinoma cases are 5,73,278 (3% of all malignancies), among them 2.1% died³. In Bangladesh, newly diagnosed urinary bladder carcinoma cases are 1,626 (1.1% of all malignancies), among them 0.92% died. Prevalence of urinary bladder carcinoma in Bangladesh is 2.08%⁴.

Urothelial carcinoma (UC) represent more than 90% of bladder cancer ; other histological types include squamous cell carcinoma (2%) and adenocarcinoma (1.5%)⁵. Bladder cancers can also be classified as non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC)⁶.

The important factors in determining the prognosis of the bladder carcinoma are infiltrative pattern, histopathological grading, staging and tumor recurrence. However, these pathological variables are not conclusive to predict the prognosis. Newer molecular techniques are becoming day by day the important adjunct to predict the behavior of bladder carcinoma⁷.

The tumor suppressor gene *TP53* had been shown potential biological and prognostic significance of urinary bladder carcinoma⁸. *TP53* mutations cause loss of normal p53 function and accumulated DNA damage may result in tumor development⁹. Mutated *TP53* gene results in p53 protein accumulation in cells' nuclei; this accumulation is detectable with immunohistochemical methods¹⁰. Mutations of *TP53* are more common in high-grade invasive urinary bladder cancers¹¹.

Ki-67 is a nuclear protein which can be detected during the cell cycles of G1, S, G2, and M phases except G0 and can be detected by immunohistochemistry (IHC) to assess the activities of cell proliferation in various cancers¹². Nuclear Ki-67 antigen expression indicates the biological aggressiveness of a malignancy¹³. Many studies had shown a definite correlation of p53 and Ki-67 immunoexpression with histopathological grade and stage of UBC^{7,14,20,23}.

The aim of this study was to observe the immunoexpression of p53 and Ki-67 in urinary bladder carcinoma and their association with tumor grade and muscle-invasiveness.

Methods:

This cross-sectional study was conducted at the Department of Pathology, Sylhet MAG Osmani Medical College from September 2018 to July 2020. A total of 57 patients were selected following the enrolment criteria who were later underwent trans-urethral resection of bladder tumor at the Department of Urology, Sylhet MAG Osmani Medical College Hospital. These specimens were then processed and routine H&E stain was carried out to see the histologic type, tumor grade and muscle invasiveness.

The paraffin blocks were then carried to the Armed Forces Institute of Pathology (AFIP), Dhaka for immunostaining of p53 and Ki-67. Assessment of p53 and Ki67 immunohistochemical staining done according to Wang et al¹⁴ and Jawad, Ali and Kamal¹⁵ respectively. Histopathological diagnosis, p53 and Ki-67 expression were done in all of these patients.

Informed written consent was obtained from the study subjects before the commencement of the study. All data were kept confidential and used only for this study purpose. Ethical clearance was obtained from the ethical committee of Sylhet MAG Osmani Medical College.

Statistical analysis of the results was obtained by using Statistical Packages for Social Sciences (SPSS-23) software.

Results:

Age of the study patients ranged from 35 to 80 years with a mean of 60.60 ± 11.14 with male to female ratio of 4.7:1. Male patients were predominant (82.5%) and female patients were 17.5% [Table 1]. Most of the male patients in this study had the habit of cigarette smoking [Table 2]. Among the 57 urinary bladder carcinoma cases, 53 cases (93.0%) were histopathologically diagnosed as urothelial carcinoma. Adenocarcinoma and squamous cell carcinoma were 2 cases (3.5%) and 2 cases (3.5%) respectively [Figure-1]. According to WHO grading system, 31 cases (54.4%) were histopathologically diagnosed as high grade and 26 cases (45.6%) as low grade. Thirty cases (52.6%) of bladder carcinoma were muscle invasive (MIBC) and 27 cases (47.4%) were non-muscle invasive (NMIBC). The relationship of high and low grades with muscle invasiveness of urinary bladder carcinoma were statistically significant ($P < 0.001$) [Table 3]. Among the 31 high grade UBC cases, 24 cases (77.4%) showed positive p53 expression and 7 cases (22.6%) showed negative p53 expression; in contrast only 6 cases (23.1%) low grade carcinoma showed positive p53 expression and 20 cases (76.9%) showed negative p53 expression. Among 30 MIBC cases, 22 cases (73.3%) were positive for p53 expression and 8 cases (26.7%)

were negative for p53 expression; on the other hand 19 cases (70.4%) NMIBC were negative for p53 expression and 8 cases (29.6%) were positive for p53 expression. Statistically significant association ($P < 0.05$) was observed in histopathological grades and muscle invasiveness of UBC with p53 expression [Table: 4, 5 and Figure-2, 3]. In this study, negative Ki-67 expression was noted in 23 cases and positive expression in 34 patients. LG tumors showed mainly negative expression (19 cases) and HG tumors exhibited positive expression (27 cases). Among 30 MIBC cases, 25 (83.3%) were positive for Ki-67 expression and 5 (16.7%) were negative for Ki-67 expression; on the other hand 18 (66.7%) NMIBC were negative for Ki-67 expression and 9 (33.3%) were positive for Ki-67 expression. Statistically significant association ($P < 0.05$) was observed in histopathological grades and muscle invasiveness of UBC with Ki-67 expression [Table 6, 7 and Figure-4, 5].

Table 1: Distribution of the patients according to demographic variables (n=57).

Demographic variables	Frequency	Percentage
Age (years)		
○ ≤40	3	5.3
○ 41-50	13	22.8
○ 51-60	15	26.3
○ 61-70	18	31.6
○ >70	8	14.0
Mean ± SD (Min-Max)	60.60 ± 11.14 (35-80)	
Gender		
○ Male	47	82.5
○ Female	10	17.5
Occupation		
○ Farmer	30	52.6
○ Businessman	8	14.0
○ Service holder	3	5.3
○ Carpenter	1	1.8
○ Painter	2	3.5
○ Construction worker	3	5.3
○ Housewife	10	17.5
Socio-economic status		
○ Low	40	70.2
○ Middle	15	26.3
○ High	2	3.5

Table 2: Distribution of the patients according to smoking habit.

Smoking habit	Frequency	Percentage
Smoker	33	57.9
Non-smoker	24	42.1
Total	57	100.0

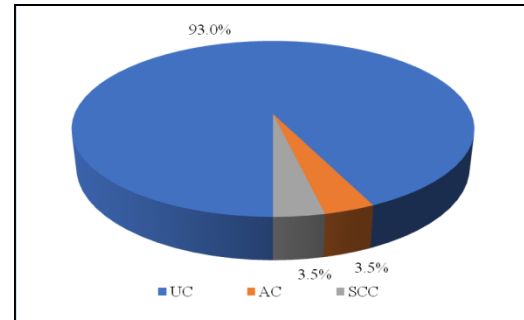


Figure 1: Pie chart showing distribution of patients according to histomorphological type (n=57).

Table 3: Relationship between histological grade and muscle invasiveness (n=57).

Muscle-invasiveness	Histopathological grade		p value*
	Low	High	
NMIBC	24 (92.3)	3 (9.7)	<0.001 ^s
MIBC	2 (7.7)	28 (90.3)	
Total	26 (100.0)	31 (100.0)	

*Chi-square test was employed to analyse the data.

Figures in parenthesis denote the corresponding percentage.

Table 4: Association of p53 expression with grade and muscle-invasiveness of urinary bladder carcinoma (n=57).

Variables	P53 expression		p value*
	Positive	Negative	
Histopathological grade			
○ Low	6 (23.1)	20 (76.9)	<0.001 ^s
○ High	24 (77.4)	7 (22.6)	
Muscle invasiveness			
○ NMIBC	8 (29.6)	19 (70.4)	0.001 ^s
○ MIBC	22 (73.3)	8 (26.7)	

*Chi-square test was employed to analyse the data.

Figures in parenthesis denote the corresponding percentage.

Table 5: Association of p53 expression score with grade and muscle-invasiveness of urinary bladder carcinoma (n=57).

Variables	p53 expression score				p value*
	0	1	2	3	
Histopathological grade					
○ Low (n=26)	20 (76.9)	2 (7.7)	3 (11.5)	1 (3.8)	<0.001 ^s
○ High (n=31)	7 (22.6)	5 (16.1)	6 (19.4)	13 (41.9)	
Muscle invasiveness					
○ NMIBC	19 (70.4)	3 (11.1)	3 (11.1)	2 (7.4)	0.005 ^s
○ MIBC	8 (26.7)	4 (13.3)	6 (20.0)	12 (40.0)	

*Chi-square test was employed to analyse the data.

Figures in parenthesis denote the corresponding percentage.

Table 6: Association of Ki-67 expression with grade and muscle-invasiveness of urinary bladder carcinoma (n=57).

Variables	Ki-67 expression		p value*
	Positive	Negative	
Histopathological grade			
○ Low	7 (26.9)	19 (73.1)	<0.001 ^s
○ High	27 (87.1)	4 (12.9)	
Muscle invasiveness			
○ NMIBC	9 (33.3)	18 (66.7)	0.001 ^s
○ MIBC	25 (83.3)	5 (16.7)	

*Chi-square test was employed to analyse the data.

Figures in parenthesis denote the corresponding percentage.

Table 7: Association of Ki-67 expression score with grade and muscle-invasiveness of urinary bladder carcinoma (n=57).

Variables		Ki-67 expression score				p value*
		0	1	2	3	
Histopathological grade						
○	Low (n=26)	19 (73.1)	3 (11.5)	3 (11.5)	1 (3.8)	<0.001
○	High (n=31)	4 (12.9)	7 (22.6)	11 (35.5)	9 (29.0)	
Muscle invasiveness						
○	NMIBC	18 (66.7)	3 (11.1)	4 (14.8)	2 (7.4)	0.005
○	MIBC	5 (16.7)	7 (23.3)	10 (33.3)	8 (26.7)	

*Chi-square test was employed to analyse the data.

Figures in parenthesis denote the corresponding percentage.

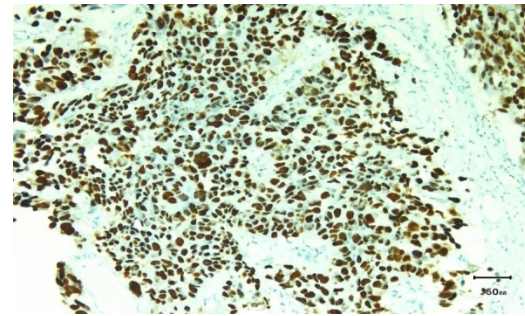


Figure 2: Photomicrograph showing positive p53 immunoeexpression (Score 3+) in high grade urothelial carcinoma, MIBC (Case no 17, 200x magnification).

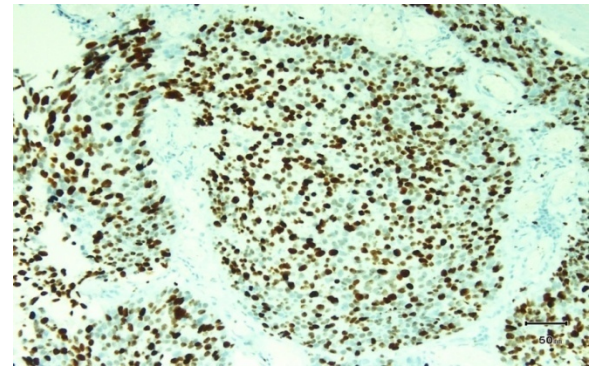


Figure 3: Photomicrograph showing positive Ki-67 immunoeexpression (Score 3+) in high grade urothelial carcinoma, MIBC (Case no 17, 200x magnification).

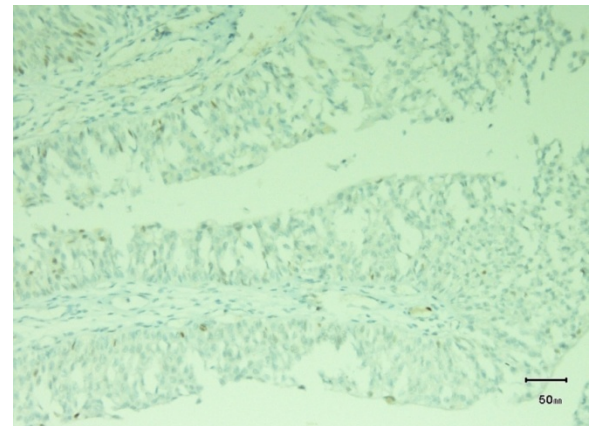


Figure 4: Photomicrograph showing negative p53 immunoeexpression (Score 0) in low grade urothelial carcinoma, NMIBC (Case no 25, 200x magnification).

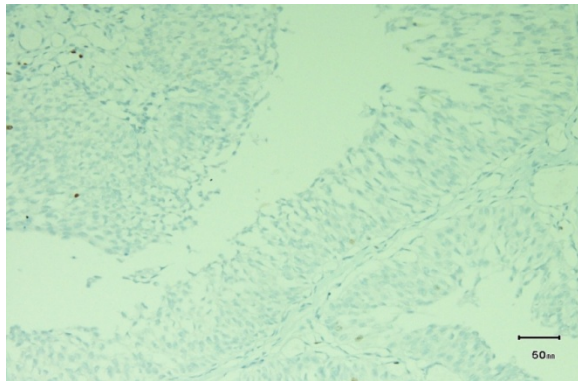


Figure 5: Photomicrograph showing negative Ki-67 immunoreactivity (Score 0) in low grade urothelial carcinoma, NMIBC (Case no 25, 200x magnification).

Discussion:

Age of the study patients ranged from 35 to 80 years with a mean of 60.60 ± 11.14 and male to female ratio was 4.7:1 which are almost similar to the findings in the study of Badruddoza et al in Bangladesh¹⁶. They found mean age of the patients were 60.00 ± 10.00 with male to female ratio of 7:1. A study done by Xu et al where mean age of the patients with neoplastic lesions was 64.3 years with male 74% and female 26%. The higher incidence of UBC in male may be due to the personal habit such as smoking and more exposure to toxic agents due to their occupation¹.

The highest number 70.2% of the patients were from low, 26.3% from middle and 3.5% from high socio economic status in the current study. Haque et al found that 62.0% of patients were from low and 38.0% from middle socio economic status¹⁷. A study by Gandomani et al found that there was a relationship between low socio-economic status and increased tendency of urinary bladder carcinoma².

In the present study, 33 (57.9%) patients were smokers and 24 (42.1%) were non smokers. Most of the male patients in this study had the habit of cigarette smoking. In Bangladesh, a study done by Haque et al found that 72% patients had smoking habit¹⁷. Chinnasamy et al revealed that most of the bladder cancer patients (71.2%) had smoking habit which is close to the finding of this study¹⁸.

Out of 57 study cases, 53 (93%) were urothelial carcinoma, 2 (3.5%) were squamous cell

carcinoma and 2 cases (3.5%) were adenocarcinoma. As all the specimens were collected by

TURBT so adenocarcinoma and squamous cell carcinoma may be glandular and squamous differentiation of urothelial carcinoma. A study performed by Thakur et al found 97.3% urothelial carcinoma followed by 1.8% squamous cell carcinoma and 0.9% adenocarcinoma⁷. Jeelani et al reported 98% urothelial carcinoma and 2% adenocarcinoma¹⁹. These findings are almost similar to this study.

All cases were classified as low grade (LG) and high grade (HG) according to WHO grading system. It was observed that 31 (54.4%) patients had high grade carcinoma and 26 (45.6%) patients had low grade carcinoma. Mohamed in his study found 79% high grade and 21% low grade tumor²⁰. Chinnasamy et al observed 63.5% high grade and 36.5% low grade carcinoma¹⁸. The findings of all these studies regarding histologic grades are almost similar to the findings of present study.

In this study, 30 cases (52.6%) were MIBC and 27 cases (47.4%) were NMIBC. Chou et al. (2013) also found 59.5% MIBC and 40.5% NMIBC²¹. On the other hand, Mohamed found 72.6% NMIBC and 25.8% MIBC²⁰. Xu et al found 69.4% NMIBC and 30.6%

MIBC¹. In current study, among 26 low grade carcinomas, 24 (92.3%) were NMIBC and 2 (7.7%) were MIBC. On the other hand, among 31 high grade carcinomas 3 (9.7%) were NMIBC and 28(90.3%) were MIBC. The relationship of different tumor grades and muscle

invasiveness was found statistically significant ($p < 0.05$). High incidence of MIBC in this study may be due to poverty, lack of knowledge, lack of urological facilities in rural health services, as well as social and religious restrictions especially for female patients which prevent them from earlier treatment opportunities. Moreover, all the study cases were from

SOMCH, which is a tertiary level hospital and patients are admitted here with advanced stage of disease and/or as referred complicated cases. Among the 57 patients, immunohistochemically 30 cases (52.6%) were p53 positive and 27

cases (47.4%) were p53 negative. Positive p53 expression was significantly higher among patients with high grade tumor as well as in patients with MIBC ($P<0.05$). Among the 31 high grade tumors, positive expression of p53 was observed in 24 cases (77.4%); whereas 6 cases (23.1%) showed positive expression of p53 in low grade tumors. Among 30 cases of MIBC, 22 cases (73.3%) were positive for p53 expression. Nassa et al found more p53-positive cases in high grade tumors than in low grade tumors and a higher percentage of muscle-invasive tumors were positively stained for p53 in comparison with NMI tumors, which is similar to this study²². Kilicli – Camur et al investigated the prognostic value of the p53 and Ki-67 immunoreactivity indices. They found more p53 immunopositivity associated with high grade and advanced stage²³. Tsuji et al found no significant association between tumor grade, stage and p53 expression. The discrepancies between studies could be related to the choice of antibody, variability in the interpretation and stratification criteria and inconsistency in specimen handling and technical procedures²⁴. In the present study, 34 cases (59.6%) were Ki-67 positive and 23 cases (40.4%) were Ki-67 negative. Among 31 high grade tumors, positive Ki67 expression was observed in 27 cases (87.1%) and 7 cases (26.9%) out of 26 low grade tumors showed positive Ki-67 expression in this study. The expression of Ki-67 was statistically significant ($p<0.05$) in high grade UBC. On the other hand, there was high percentages (73.1%) of negative Ki-67 immunoexpression in the low grade tumors. Haque et al found statistically significant ($p<0.05$) expression of Ki67 in high grade UBC¹⁷. A study performed by Jawad, Ali and Kamal found 84% high grade and 15.0% low grade urinary bladder carcinoma with positive Ki-67 expression; which is almost similar to this study¹⁵. Similar findings were also observed in the study performed by Margulis et al¹³. Among 30 cases of MIBC, 25 cases (83.3%) were positive for Ki-67 expression. On the other hand 9 cases (33.3%) out of 27 NMIBC showed positive Ki-67 expression. These findings are also supported by the studies performed by Margulis et al and Jawad, Ali and Kamal^{13,15}.

Conclusion: The current study was intended to find out the role of p53 and Ki-67 immunoexpressions in tumor grades and muscle-invasiveness of urinary bladder carcinoma, which can help in predicting the clinical outcome. It was found that high expression of p53 and Ki-67 was closely related to higher grades and muscle-invasiveness of tumors. The use of p53 and Ki-67 immunomarkers in conjunction with histopathological grading and staging may provide significant prognostic information for categorizing high risk patients in urinary bladder carcinoma.

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