Expression of p53 in Urothelial Carcinoma of the Urinary Bladder: Correlation with Tumor Grade and Muscle Invasion

Azim T,[1] Jinnah MS,[2] Aktar M,[3] Ahmed N,[4] *Badhan RE[5]

Abstract

Background: The p53 gene, a well-known tumor suppressor gene located on chromosome 17p13.1, plays a pivotal role in regulating cell cycle arrest and apoptosis. Mutations or overexpression of p53 have been linked to tumor progression in urothelial carcinoma.

Aim: To evaluate p53 expression in urothelial carcinoma of the urinary bladder and determine its relationship with histopathological grade and muscle invasion.

Methods: This cross-sectional study was conducted at Dhaka Medical College between March 2020 and February 2022. Forty histopathologically diagnosed urothelial carcinoma cases from TURBT or cystectomy specimens were included. p53 expression was assessed by immunohistochemistry and correlated with tumor grade and the presence of muscle invasion.

Results: The mean age was 60.85 ± 11.18 years (range 40–85), with a male-to-female ratio of 3.4:1. p53 was positive in 65% of all cases and in 92.3% of high-grade tumors, while most low-grade tumors were negative. Among high-grade, muscle-invasive cases, p53 positivity was observed in 93.3% of cases. The correlation between p53 overexpression and both tumor grade and muscle invasion was statistically significant ($p<0.05$).

Conclusion: p53 expression is significantly associated with aggressive urothelial carcinoma phenotypes, supporting its utility as a prognostic biomarker in clinical decision-making.

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1. *Dr. Tasmia Azim, Assistant Professor, Department of Pathology, Holy Family Red Crescent Medical College, Dhaka. tasmia.azim@gmail.com*
2. *Professor Dr. Mohammed Shahed Ali Jinnah. Ex-director, National Institute of Laboratory Medicine and Referral Centre (NILMRC), Dhaka. shahed.jinnah63@gmail.com*
3. *Dr. Marufa Aktar, Assistant Professor, Department of Pathology, Sher E Bangla Medical College, Barishal. marufaepu@gmail.com*
4. *Dr. Nasim Ahmed, Junior Consultant,Department of Anesthesia, Analgesia, Palliative and Intensive Care Medicine, Dhaka Medical College and Hospital. : dr.ahmednasim@gmail.com*
5. **Dr. Raisa Enayet Badhan, Medical Officer, Department of Microbiology, National Institute of Burn and Plastic Surgery, Dhaka. raisabadhan@gmail.com*

**For Correspondence*

Introduction

Bladder cancer ranks among the most prevalent malignancies globally, with urothelial carcinoma accounting for the vast majority of cases.[1] Although approximately 70% of patients present with non-muscle-invasive bladder carcinoma (NMIBC), recurrence and progression rates remain high, with up to 15% eventually developing muscle-invasive disease.[2,3] Prognosis in muscle-invasive bladder carcinoma (MIBC) remains poor, highlighting the need for reliable biomarkers that can guide clinical management.

The tumor suppressor gene TP53, located on chromosome 17p13.1, encodes a nuclear phosphoprotein critical for cell cycle regulation at the G1/S checkpoint.[4] In its wild-type form, p53 halts the cell cycle to facilitate DNA repair or initiate apoptosis. Mutations in TP53 disrupt this safeguard, enabling genomic instability and tumor progression. Immunohistochemical detection of p53 overexpression often reflects underlying TP53 mutations and has been correlated with higher-grade and stage tumors in urothelial carcinoma.[5,6]

Considering the clinical significance of p53 alterations in bladder cancer, this study aimed to evaluate p53 expression in urothelial carcinoma of the urinary bladder and to investigate its association with tumor grade and muscle invasion.

Methods

Study Design

A cross-sectional study was conducted over two years (March 2020 – February 2022) in the Department of Pathology, Dhaka Medical College, following approval from the institutional ethics committee.

Study Population

Forty histopathologically confirmed urothelial carcinoma cases from TURBT or cystectomy specimens were enrolled using consecutive sampling. All age groups and both sexes were included. Exclusion criteria were prior chemo/radiotherapy and inadequate tissue preservation.

Forty histopathologically confirmed cases of urothelial carcinoma obtained from TURBT and cystectomy specimens were enrolled using consecutive sampling.

Presence of muscularis propria was specifically assessed in all specimens. Muscularis propria was not identified in a proportion of TURBT specimens, predominantly in cases diagnosed as low-grade urothelial carcinoma, as these tumors were often resected superficially.

High-grade urothelial carcinoma cases were more frequently accompanied by muscularis propria, allowing reliable assessment of muscle invasion in this subgroup.

Cases lacking muscularis propria were excluded from muscle invasion analysis but were included for evaluation of tumor grade and p53 immunoexpression.

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Sample Size

Sample size determination is done by the following formula:

$$n = \frac{Z^2(p \times q)}{d^2}$$

Here,

n = Sample size

z = Standard normal deviation. Usually assumed at 1.96 which corresponds to 95% confidence limit

p = Prevalence in target population. 5 year prevalence in Bangladesh is 2.3% = 0.023[6]
q = 1-p = 1-0.023 = 0.977
d = Accepted standard error = 0.05

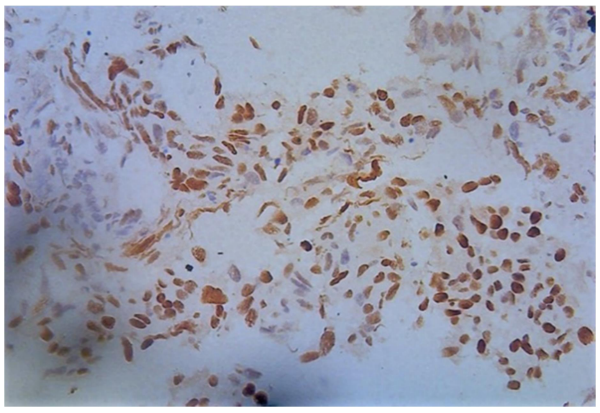
Thus, $\frac{(0.023)(0.977)(1.96)^2}{0.05^2} = 34.53$
Calculating the above formula sample size was 35 (estimated sample size).
A total of 40 samples were enrolled in this study.

Data Parameters
Recorded parameters included demographic details, clinical presentation, smoking status, tumor location, histological grade, and presence or absence of muscle invasion.

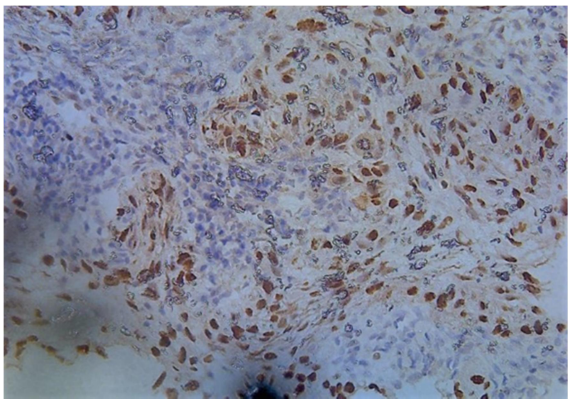
Immunohistochemistry for P53
Sections of 5 µm thickness from representative paraffin blocks were processed using standard deparaffinization and rehydration techniques. Antigen retrieval was achieved with Dako Target Retrieval Solution in a water bath at 95–99 °C for 30–40 minutes. The primary antibody used was Monoclonal Mouse Anti-Human p53 protein (clone DO-7, Dako). Detection utilized the DAKO REAL™ EnVision™ HRP system. Known p53-positive ovarian tumor sections served as the positive control.

Immunostaining Procedure for p53
Formalin-fixed, paraffin-embedded tissue sections (4.0 µm) were mounted on poly-L-lysine-coated slides and processed for immunohistochemical staining of p53. After deparaffinization, rehydration, and antigen retrieval, slides were washed with TBS and subjected to automated staining, including incubation with primary antibody, EnVision detection, DAB chromogen, and hematoxylin counterstaining. Finally, slides were dehydrated, cleared in xylene, and mounted with DPX.

Immunoreactivity Scoring Criteria
Only nuclear staining of urothelial tumor cells was evaluated. Positive expression was defined as >10% nuclear immunoreactivity, while ≤10% staining was regarded as negative.[7] Two independent observers evaluated all cases without knowledge of clinicopathological parameters. (Figure 1)



(a)



(b)

Figure 1. (a) Photomicrograph showing high-grade urothelial carcinoma with positive staining for p53 (case no. 36, IHC staining, X 400x) (b) Photomicrograph showing high-grade muscle-invasive urothelial carcinoma with positive staining for p53 (case no. 23, IHC staining, X 400x).

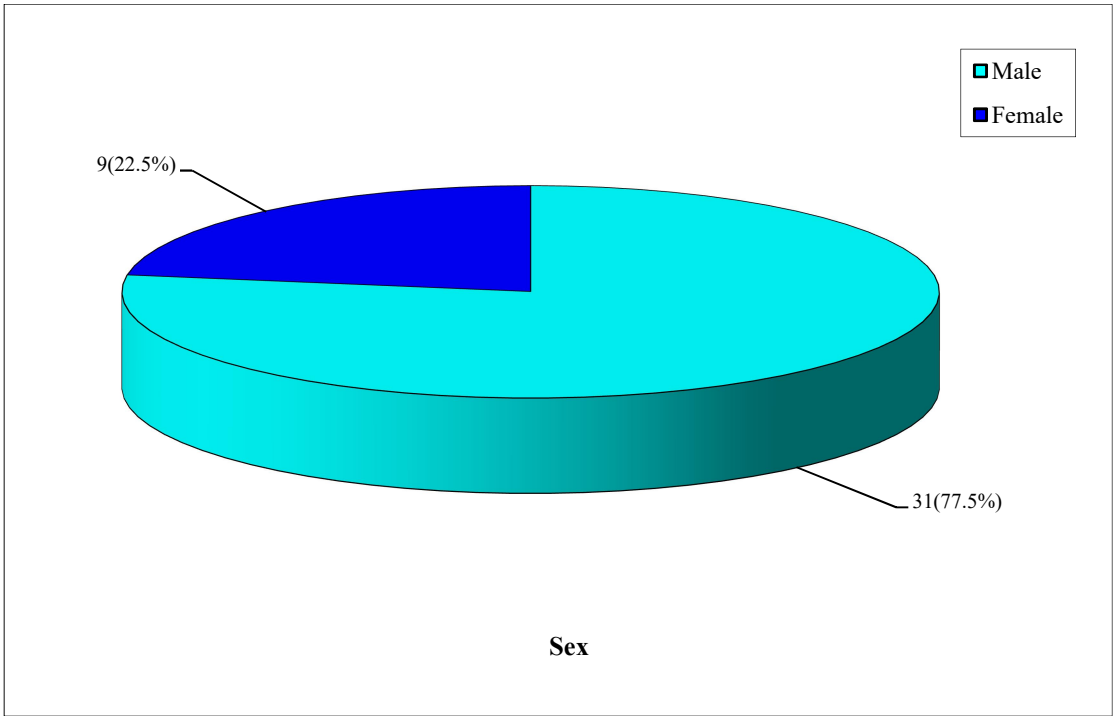


Figure 2. Pie chart showing distribution of the study patients according to sex (n=40)

Statistical Analysis
All statistical procedures were performed in SPSS v22. Associations between p53 expression, histological grade, and muscle invasion were tested using the Chi-square test, with p-values <0.05 deemed significant.

Results
Histopathologically diagnosed 40 cases of urothelial carcinoma were included in this study. After assessing the grade of the tumor and muscle invasion immunohistochemical study of p53 was done in all 40 cases.

The age distribution of the patients ranged from 40 to 85 years. Most of the patients (35%) were found to be in between 61 to 70 years. The mean age was 60.85±11.18 years (Table I).
Figure 2 shows the distribution of the study patients by sex. It was observed that 31 (77.5%) patients were male and 9 (22.5%) were female.

Table I: Distribution of study patients according to sex (n=40)

Age group (years)	Number of patients (n)	Percentage (%)
≤ 40	2	5.0
41 – 50	7	17.5
51 – 60	11	27.5
61 – 70	14	35.0
71 – 80	4	10.0
> 80	2	5.0
Total	40	100.0

Out of 40 cases, more than half (52.5%) of patients had only haematuria, 13 (32.5%) had haematuria with lower urinary tract symptoms (LUTS), and 6 (15.0%) had haematuria with lower abdominal pain. 31 patients (77.5%) were smokers and 9 (22.5%) were non-smokers.

Out of 40 cases, 18 (45.0%) patients had a tumor on the lateral wall, 9 (22.5%) had a

tumor on the anterior wall, and 13 (32.5%) had a tumor on the posterior wall. The tumor location was assessed according to cystoscopic findings. Among 40 cases of urothelial carcinoma, 36 cases (90.0%) were TURBT specimens and 4 cases (10.0%) were cystectomy specimens.

Table II shows that, out of 40 cases, 26 (65.0%) patients were reported as high-grade urothelial carcinoma, and 14 (35.0%) patients were reported as low-grade urothelial carcinoma. Among 40 cases, more than one-third (37.5%) of patients had muscle-invasive bladder cancer (MIBC), and 25 (62.5%) patients had non-muscle-invasive bladder cancer (NMIBC).

Table II shows that, among 40 cases, 26 (65.0%) patients exhibited positive p53 expression, and 14 (35.0%) cases showed negative p53 expression.

Table II: Distributions of the study patients according to tumor grade and muscle invasion (n=40)

	Number	Percentage
Grade		
High grade	26	65.0
Low grade	14	35.0
Muscle invasion		
Present	15	37.5
Absent	25	62.5

Table III: Distributions of the study patients according to p53 expression (n=40)

	Number	Percentage
p53 expression		
Positive	26	65.0
Negative	14	35.0

Table IV: Association of histopathological grade with muscle invasion of the study patients (n=40)

Muscle invasion	Histopathological grade				*p* value
	High grade (n=26)		Low grade (n=14)		
	n	%	n	%	
Present	15	57.7	0	0.0	0.001[s]
Absent	11	42.3	14	100.0	

s=significant; p value reached from Chi-square test

Table V: Association of p53 expression with histopathological grade in study patients (n=40)

	Histopathological grade				*p* value
	High grade (n=26)		Low grade (n=14)		
	N	%	n	%	
p53 expression					
Positive	24	92.3	2	14.3	0.001[s]
Negative	2	7.7	12	85.7	

s=significant; P value reached from Chi-square test

Table VI: Association of p53 expression with muscle invasion of the study patients (n=40)

	Muscle invasion				*p* value
	Present (n=15)		Absent (n=25)		
	n	%	n	%	
p53 expression					
Positive	14	93.3	12	48.0	0.004[s]
Negative	1	6.7	13	52.0	

s=significant; P value reached from Chi-square test

The majority of the patients (57.7%) with high-grade urothelial carcinoma were muscle invasive, and muscle invasion was absent in all 14 (100%) patients with low-grade urothelial carcinoma. The difference was statistically significant ($p<0.05$). (Table IV)

Table V shows that, 92.3% of high-grade urothelial carcinoma cases showed positive p53 expression, and only 2 (14.3%) cases out of 14 cases with low-grade urothelial carcinoma showed positive p53 expression. More than half (53.8%) of patients with high-grade urothelial carcinoma showed positive expression for p53. The difference was statistically significant ($p<0.05$).

Among 40 cases, 15 cases were muscle-invasive urothelial carcinoma. Among the cases 93.3% patients had positive p53 expression. The rest were non-muscle invasive urothelial carcinoma. The 52.0% patients had negative p53 expression. The difference was statistically significant ($p<0.05$). (Table 6)

Discussion

This cross-sectional study was carried out with the aim of seeing the histomorphological distribution of urothelial carcinoma of the urinary bladder and to detect the pattern of p53 expression in urothelial carcinoma of the urinary bladder and their association with histopathological grade and muscle invasion. Regarding the histopathological diagnosis, about 65.0% (26/40) of patients had high-grade urothelial carcinoma, and the rest (14/40) had low-grade urothelial carcinoma. More than one third (37.5%) (15/40) patients had muscle-invasive bladder tumors. Sahoo et al. (2021) study observed 52.7% (19/36) of patients had high-grade and 47.2% (17/36) had low-grade urothelial carcinoma, which supports the present study.[8] Similarly, Goyal et al. (2014) and Senturk et al. (2010) also found predominance of high-grade bladder tumors.[9,10] Bahadir et al. (2009) study found that 18.80% was high grade and 83.80% was low grade, which differs from the present study.[11] Hamdi, (2018) study found that histologically, there were 2.0% cases of papilloma, 22.0% cases of papillary urothelial neoplasm of low malignant potential (PUNLMP), 46.0% cases of low-grade carcinoma, and 30.0% cases of high-grade carcinoma, which differ from the present study.[12] Yalcin et al. (2015) study observed 58 cases diagnosed with primary superficial bladder cancer, out of which 58.6% had a diagnosis of non-invasive papillary urothelial carcinoma, low grade, 17.6% had non-invasive papillary urothelial carcinoma, high grade, 6.9% had invasive papillary urothelial carcinoma, low grade, 5.2% had invasive papillary urothelial carcinoma, high grade and 12.1% had other diagnosis.[13]

Regarding p53 expression, Stein et al. (1998) reported that the tumor suppressor gene p53 plays an important role in cell cycle regulation.[14] A combination of immunohistochemical p53 protein detection and molecular sequence analysis has shown that p53 protein accumulation correlates with the amount of mutant p53 gene.[15] In this current study, it was observed that 65.0% patients had positive p53 expression and 35.0% had negative p53 expression. Bakir et al. (2011) study mentioned that the main reason for the high p53 positive expression in bladder cancers is the formation of dysfunctional mutated p53; for that reason, the higher the p53 in bladder cancer, the higher the mutated p53, leading to less functional wild p53.[16] Black and Dinney (2007) study mentioned that the process of angiogenesis helps increase the tumor size and facilitates metastasis.[17] Dysfunction of p53 also gives rise to angiogenesis and hence metastasis. Thus, in bladder cancers, the overexpression of p53 represents the accumulation of shifted, dysfunctional

interpretation of the protein, leading to a state of high rate proliferation, immunosuppression, which all increase the invasiveness and aggressiveness of the excrescence as found in Bakir et al. (2011) study.[16] Kalantari and Ahmadnia's (2007) study showed 33/50 cases (66%) were p53 positive, which corresponds with the present study.[18] Camur et al. 2002 also showed 58.47% p53-positive cases, which is similar to the current study. Senturk et al. (2010) and Kong et al. (1998) studies found that p53 expression was positive in 47.6% (40/84) and 45.0% (44/89) cases, respectively, which are less than the present study.[4,10] In Bangladesh, a study done by Biswas S (2019) found a significant difference in the expression of p53 in different grades and stages of urothelial carcinoma.[19] A study by Jannat AD (2020) also found 26/50 cases (52%) positive for p53 expression, which supports the current study.[20]

Regarding the association between histopathological grade and p53 expression in this present study, it was observed that 92.3% of high-grade tumors and 14.3% of low-grade tumors had positive p53 expression. Positive p53 expression was significantly (p<0.05) higher in high-grade. Kalantari and Ahmadnia, (2007) study found that 85.0% of the high-grade carcinomas showed >10% p53 expression.[18] Sahoo et al. (2021) study showed 66% cases of high-grade carcinomas and 20.0% cases of low-grade carcinomas with >10% p53 expression.[8] Similar findings were also found by Sen et al. (2016).[21] Increasing histological grade is associated with increased p53 expression observed by many investigators. Korkolopoulou et al. (2002) stated that the observance of p53 expression in advanced stages supports a crucial role for p53 mutations in bladder cancer progression.[22] Although there is an undisputed relationship between p53 positivity and high histological grade, p53 expression can decrease in retrospective studies as paraffin-embedded tissues may lose their immunoreactivity with time. Senturk et al. (2010) study found that increased p53 expression with increased histological grade and tumor invasion also supports the role of p53 mutations in urothelial carcinoma progression.[10]

The study was performed to assess the risk stratification of the patients, to predict the prognosis of the patient, as well as to guide therapeutic strategies and improve the outcome of the patient.

The findings of this study highlight the importance of p53 expression in urothelial carcinoma of the urinary bladder, but several limitations must be considered. As this was a single-center, cross-sectional observational study, the results may not represent the true nationwide distribution, and the absence of follow-up restricted assessment of patient outcomes in relation to p53 expression. Nevertheless, the results point toward the potential utility of p53 immunomarker in detecting aggressive tumors and identifying patients at high risk. To establish stronger evidence, multicenter studies with standardized immunohistochemical and molecular methods are recommended, along with long-term follow-up to correlate p53 overexpression with disease progression, recurrence, and survival.

Conclusion

This study demonstrated that p53 expression correlates strongly with tumor grade and muscle invasiveness in urothelial carcinoma. As an indicator of genomic instability and aggressive tumor behavior, p53 immunoexpression may serve as an **adjunctive biomarker** associated with tumor aggressiveness beyond routine histopathological evaluation. Its role in tumor biology further emphasizes the importance of

p53 as both a diagnostic and prognostic marker, aiding in the identification of patients with poor outcomes who may benefit from closer follow-up and advanced therapeutic strategies.

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