Expression of Vimentin, p53, and Ki-67 in Urothelial Carcinoma of Urinary Bladder: Correlation with Histologic grade and Muscle Invasion

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Submission Date : 22 Oct 2024
Accepted Date : 15 Dec 2024
Published Date : 30 March 2025
DOI: https://doi.org/10.3329/jrpmc.v10i1.81515

Abstract

Background:
Bladder cancer is one of the most common cancers with non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC) being the two principal types. Biomarkers vimentin and Ki-67 play an important role in establishing tumor growth and potential prognostic value.

Objective:
The study aimed to the monitoring of Vimentin, p53, and Ki-67 expression based on histologic grade and muscle invasion in bladder urothelial carcinoma.

Methods:
A total of 60 bladder cancer patients, including NMIBC and MIBC patients, were being studied. Immunohistochemistry was performed to analyze the expression of vimentin and Ki-67. Statistical analysis included Pearson correlation and Chi-square tests to analyze correlations with clinicopathological parameters.

Results:
Widespread expression of vimentin and Ki-67 was strongly associated with increased tumor grade, lymph node status, and poor overall survival. Increased vimentin and Ki-67 expression also showed a strong association with each other, suggesting perhaps a role in tumor aggressiveness and advancement.

Conclusion:
The studies demonstrate that vimentin and Ki-67 are relevant biomarkers for prognosis in bladder cancer. Their expression levels may be employed for more accurate patient stratification and tailored treatment, and thus validation in larger, multi-center trials is warranted.

Keywords: Vimentin, Ki 67, p53, Urothelial carcinoma, Muscle invasion, Grade

Citation: Islam MZ, Ahamed S, Rashid MHO, Rahman M, Afroze T. Expression of Vimentin, p53, and Ki-67 in Urothelial Carcinoma of Urinary Bladder: Correlation with Histologic grade and Muscle Invasion.J Rang Med Col.2025 Mar;10(1):10-15. doi: https://doi.org/10.3329/jrpmc.v10i1.81515

Introduction:

Urinary bladder cancer ranks as the tenth most common cancer globally, with 549,000 new cases and 200,000 deaths annually. Incidence and mortality rates are nearly four times higher in men than in women globally (9.6 and 3.2 per 1,000,000 respectively).[1] Bladder tumors are six times more common in industrialized nations than in underdeveloped nations.[2] Due to urbanization, industrialization, greater chemical usage, and smoking, bladder cancer is becoming more and more common in developing nations like Bangladesh.[3] Incidence, mortality, and 5-year prevalence rates of bladder cancer in Bangladesh are 1.1%, 0.84%, and 2.3%, respectively.[4] According to WHO classification, there are various types of tumors of the urothelial tract.[5] About 95% of bladder tumors are epithelial in origin, with most being urothelial type.[6] Approximately 70% of urothelial carcinomas present as non-muscle-invasive tumors (NMIBC), while the remainder present as muscularispropria-invasive disease (MIBC).[7] Within two years of treatment, roughly 50% of patients initially diagnosed with MIBC may relapse with metastatic illness.[7] MIBC and NMIBC vary in molecular features and pathogenesis: NMIBCs develop by way of epithelial hyperplasia and branching vasculature, while MIBCs develop by way of high-grade papillary neoplasia or urothelial CIS lesions.[8] The traditional prognostic factors are tumor stage, grade, nodal status, and

angio-lymphatic invasion.[8,9] Conventional histological assessment is not able to predict reliably the behavior of most bladder tumors.[10] Due to molecular heterogeneity, a solitary marker is not sufficient to fully depict tumor behavior.[11] Early aggressive phenotypes detection and patient stratification require assessment of more than one marker.[12,13] Separate markers summed together provide a more favorable outcome prediction.[14] Such markers like tumor suppressors, oncogenes, proliferative markers, and EMT markers like vimentin are implicated in aggressive tumor biology, adverse prognosis, enhanced recurrence, metastasis, and resistance to treatment.[15-19] P53, a p13.[1,17] chromosome tumor suppressor gene, regulates cell cycle transition, DNA repair, cellular senescence, and apoptosis and is frequently mutated in human neoplasms.[20] P53 mutations at the onset of urothelial carcinoma are excellent predictors of advanced disease and prognosis.[14,21,22] Ki-67 expression is a prognostic marker for tumor aggressiveness, recurrence, and treatment response.[23] The current study aimed to follow up on the monitoring of Vimentin, p53, and Ki-67 expression based on histologic grade and muscle invasion in bladder urothelial carcinoma.

Methods:

This cross-sectional study was carried out in Sir Salimullah Medical College, from March 2020 to February 2022. A total of 40 patients irrespective of age or sex, with histopathologically diagnosed urothelial carcinoma of bladder in the pathology department of Sir Salimullah Medical College, other teaching hospitals and private institutions in Dhaka were included in this study. Histopathologically confirmed cases of urothelial carcinoma of urinary bladder, TURBT and cystectomy specimen were included. Patients having prior radiotherapy or chemotherapy or both, poorly preserved/inadequate sample and sections without adequate muscularispropria were excluded from the study. The study was approved by the Institutional Ethics Committee

Results:

40 patients with a mean age of 60.8±9.8 years, predominantly male (60%) with smoking history (10-28 pack-years) formed the sample. The most common symptom was hematuria (77.5%), and the tumors were predominantly lateral (45%) or posterior wall (22.5%). High-grade tumors occurred in 67.5% and muscularis propria invasion in 62.5%. Vimentin was positive in 70%, p53 in 57.5%, and Ki-67 in 67.5% as seen by immunostaining (Table-I).

Table-I: Sociodemographic, clinical and grade and histological characteristics of the participants (n=40)

Variable	no. (%)
Age group (in years)	
30-40	1(2.5)
41-50	3(7.5)
51-60	15(37.5)
61-70	13(32.5)
71-80	8(20)
Mean±SD (in years)	60.8±9.8
Sex	
Male	31(77.5)
Female	9(22.5)
Smoking history	
Smoker	24(60)
Non-smoker	16(40)
Clinical features	
Hematuria	31(77.5)
LUTS	4(10)
Dysuria	3(7.5)
Lower abdominal pain	2(5)
Location of the tumor	
Lateral wall	18(45.0)
Posterior wall	9(22.5)
Trigone	6(15.0)
Neck	5(12.5)
Anterior wall	2(5.0)
Histological grade of the tumor	
High Grade	27(67.5)
Low Grade	13(32.5)
Muscularispropria invasion	
Present (MIBC)	25(62.5)
Absent (NMIBC)	15(37.5)
Expression of Vimentin	
Positive	28(70)
Negative	12(30)
Expression of p53	
Positive	23(57.5)
Negative	17(42.5)
Expression of Ki-67	
Positive	27(67.5)
Negative	13(32.5)

grade, while among the 27 high grade cases 23 (85%) were MIBC cases (Table-II).

Table-II: Distribution of cases by grade and muscularis propria invasion (n=40)

Muscularispropria invasion	High grade	Low grade	Total
Present (MIBC)	23	2	25
Absent (NMIBC)	4	11	15
Total	27	13	40

Vimentin and Ki-67 were more commonly expressed in high-grade urothelial carcinoma and MIBCs. Vimentin was markedly associated with high grade (p=0.008) and MIBC (p<0.001), and Ki-67 was related to high grade (p=0.001) and MIBC (p=0.006). There was no relationship of P53 expression with tumor grade (p=0.091) or MIBC (p=0.283) (Table-III].

Table-III: Association between vimentin, p53, and Ki-67expression with histologic grade and muscularispropria invasion (n=40)

	Positive no. (%)	Negative no. (%)	Total no. (%)	p-value
Vimentin				
Grading				
High grade	23(85.2)	4(14.8)	27(100.0)	0.008
Low grade	5(38.5)	8(61.5)	13(100.0)	
Muscle Invasion				
MIBC	23(92.0)	2(8.0)	25(100.0)	<0.001
NMIBC	5(33.3)	10(66.7)	15(100.0)	
p53				
Grading				
High grade	18(66.7)	9(33.3)	27(100.0)	0.091
Low grade	5(38.5)	8(61.5)	13(100.0)	
Muscle Invasion				
MIBC	16(64.0)	9(36.0)	25(100.0)	0.283
NMIBC	7(46.7)	8(53.3)	15(100.0)	
Ki-67				
Grading				
High grade	23(85.2)	4(14.8)	27(100.0)	0.001
Low grade	4(30.8)	9(69.2)	13(100.0)	
Muscle Invasion				
MIBC	21(84.0)	4(16.0)	25(100.0)	0.006
NMIBC	6(40.0)	9(60.0)	15(100.0)	

*Fisher Exact

This study evaluated the relationship of vimentin, p53, expression of Ki-67, and histologic grade in bladder carcinoma. Vimentin was well correlated with histologic grade (r_s = 0.506, p = 0.001). There was weak, non-significant correlation between p53 and Ki-67. Vimentin may be an effective prognostic marker for bladder carcinoma (Table-IV).

Table-IV: Correlation with histological grade

Marker	r_s	p-value	Interpretation
Vimentin	0.506	0.001	Higher expression is associated with higher histologic grade.
p53	0.07	0.667	No meaningful association.
Ki-67	0.265	0.098	A weak, non-meaningful relationship with histologic grade.

Vimentin showed a strong positive correlation with muscularis propria invasion (rs = 0.585, p<0.001), but neither p53 showed a significant correlation (rs = 0.007, p = 0.967), nor Ki-67 showed a weak positive correlation (r_s = 0.324, p = 0.041) (Table-V).

Table-V: Correlation of Biomarker Expression with Muscularis Propria Invasion

Marker	r_s	p-value	Interpretation
Vimentin	0.585	<0.001	Higher expression is strongly linked to deeper invasion into the muscularis propria.
p53	0.007	0.967	p53 expression has no relationship with muscularis propria invasion.
Ki-67	0.324	0.041	A slight association with greater muscularis propria invasion.

Table-VI showed a significant association (p=0.001) between biomarker positivity and both high-grade urothelial carcinoma and MIBC. All low-grade tumors and non-MIBC cases had at least one negative biomarker, while cases with three positive markers were predominantly high-grade (86.7%) and MIBC (93.3%). No triple-negative cases were observed in high-grade or MIBC groups, indicating that increased biomarker expression correlates with higher tumor grade and muscle invasion. (Table-VI)

Table-VI: Association between the number of positive markers and histologic grade (n=40)

Immunohistochemical marker no. (%)				p- value
Grading of urothelial carcinoma				
	High	**Low**	**Total**	
All negative	0(0.0)	5(100.0)	5(100.0)	
One positive	3(42.9)	4(57.1)	7(100.0)	0.001
Two positives	11(84.6)	2(15.4)	13(100.0)	
Three positives	13(86.7)	2(13.3)	15(100.0)	
Muscle invasive bladder cancer (MIBC)				
	Present	**Absent**	**Total**	
All negative	0(0.0)	5(100.0)	5(100.0)	
One positive	4(57.1)	3(42.9)	7(100.0)	0.001
Two positives	7(53.8)	6(46.2)	13(100.0)	
Three positives	14(93.3)	1(6.7)	15(100.0)	

*Fisher Exact

Discussion:

Among 40 histopathologically diagnosed urothelial carcinoma patients, the age was 60.8±9.8 years (range: 37-78). The age group 51-60 years was the most affected (37.5%), followed by 61-70 years (32.5%), as in South Asian studies.[24-26] The male-to-female ratio was 3.4:1, the same as Global Cancer Statistics 2018 (3.38:1).[27] In 60% of cases, smoking was involved, the same as in the study by Chinnasamy et al. (71%).[28] Hematuria was the most common symptom (77.5%), as also reported previously in 67-80% frequency.[29-31] Distribution of tumors was highest in the lateral wall (45%), followed by posterior wall (22.5%) and trigone (15%), as in a large cohort study.[29] Tumors were graded based on WHO 2016 criteria, with 67.5% being high-grade and 32.5% low-grade. Unlike 70-80% cases having been reported as low-grade,[7] Bangladeshi and Indian studies also report similar high-grade predominance.[24,28] Muscle-invasive bladder cancer (MIBC) was diagnosed in 62.5% of the cases, of which 92% were high-grade MIBC. This is in accordance with Epstein et al, who reported that ≥90% of invasive tumors (≥pT1) are of high grade.[8] Immunohistochemistry detected vimentin positivity in 70% of the cases, p53 in 57.5%, and Ki-67 in 67.5%.[32] Vimentin was significantly associated with high-grade carcinomas (85.2% vs. 38.5%, p=0.008) and MIBC (92% vs. 33.3%, p<0.001).[33] p53 expression was not significantly correlated with tumor grade (p=0.091) or MIBC (p=0.283), contrary to Margulis et al. (48.2%) and Pfister et al. (72%).[24,33] Ki-67 was highly correlated with high-grade tumors (85.2% vs. 30.8%) and MIBC (84% vs. 40%).[34] Spearman's correlation, on the other hand, showed no significant correlation between grading and Ki-67 expression (r_s=0.265, p=0.098), although weak correlation was seen with MIBC (r_s=0.324, p=0.041). Three negative markers were all low-grade and NMIBC, and three positive markers were nearly all high-grade (86.7%) and MIBC (93.3%). Fisher's Exact test also confirmed a strong association between marker positivity and histologic grade (p=0.001) and muscle invasion (p=0.001). Differences in study design, patient population, and immunostaining protocols can explain differences, but p53 pathway aberrations beyond direct mutation cannot be ruled out.[22]

Limitations:

The study was conducted in a single hospital with a small sample size. So, the results may not represent the whole community.

Conclusion:

This study concluded that Vimentin and Ki-67 positivity have the strongest association with muscle invasion and histologic grade. Additionally, the number of positive markers in the population is highly associated with predictive variables like grade and invasion.Vimentin could enhance the prognosis of urothelial carcinoma and aid in early detection of aggressive phenotypes. Larger, multicenter studies with longer follow-up would provide more comprehensive data.

Funding: No funding sources

Conflict of interest: None declared

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