Barind Medical College Journal
Abbreviated Key Title: BMCJ
ISSN: 2518-3249 (Print)
https://bmcj.org/index.php/bmcj
Volume-10 | Issue-2 | July-Dec, 2024 | DOI: https://doi.org/10.70818/bmcj.2024.v010i02.019

OPEN ACCESS

Original Research Article

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

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Article History
Received: 10.10.2024
Accepted: 21.11.2024
Published: 31.12.2024

Abstract: ***Background*:** The incidence and prevalence of bladder cancer in developing countries like Bangladesh are rising, with a 5-year prevalence rate of 2.3%. ***Objective:*** To observe the expression of Vimentin and Ki-67 in relation to histologic grade and muscle invasion in urothelial carcinoma of the urinary bladder. ***Method:*** This prospective, cross-sectional study was conducted from March 2020 to February 2022 at Sir Salimullah Medical College and other institutions in Dhaka. Forty histopathologically confirmed urothelial carcinoma cases were included. Cases with prior radiotherapy/chemotherapy or inadequate specimens were excluded. Vimentin and Ki-67 expression were analyzed in relation to histologic grade and muscle invasion, and statistical significance was assessed using Fisher's Exact Test and Spearman correlation. ***Results:*** A total of 40 patients, the mean age was 60.8±9.8 years (range: 37-78); 77.5% presented with hematuria. Male to female ratio was 3.4:1. High-grade tumors (n=27) showed 85.2% Vimentin and 85.2% Ki-67 positivity, significantly higher than low-grade tumors (38.5% Vimentin, 30.8% Ki-67; p=0.008). Muscle-invasive bladder carcinomas (MIBC; n=25) showed 92.0% Vimentin and 84.0% Ki-67 positivity, significantly more than non-muscle-invasive bladder carcinomas (NMIBC; 33.3% Vimentin, 40.0% Ki-67; p<0.001). A weak correlation was observed between Vimentin and Ki-67 expression with MIBCs (rs=0.324, p=0.041). ***Conclusion:*** Vimentin and Ki-67 expressions are significantly associated with histologic grade and muscle invasion, indicating their potential prognostic value in urothelial carcinoma of the bladder.

Keywords: Vimentin, Ki-67, Urothelial Carcinoma, Muscle Invasion

Cite this as: Islam MZ, Ray S, Rashid MHO, Rahman M, Afroza T. Expression of Vimentin and Ki-67 in Urothelial Carcinoma of Urinary Bladder: Correlation with Histologic grade and Muscle Invasion. BMCJ. 2024;10(2):60-67.

Introduction

Urinary bladder cancer stands as the tenth most common kind of cancer across the globe, with an approximate 5,49,000 new cases and 2,00,000 mortality annually. Incidence and mortality rates are almost four times higher for men than for women all over the world, with incidence and mortality rates for men exceeding 9.6 and 3.2 per 1,000,000, respectively.[1] As per Kirkali *et al*., bladder tumors are commonly six times more common in industrialized nations than in underdeveloped ones.[2] Because of urbanization, urinary bladder cancer is becoming increasingly common in developing nations like Bangladesh, even if its

incidence and prevalence are higher in developed nations.[3] In Bangladesh, bladder cancer incidence, rate is 1.1%.[4] Urothelial neoplasms, squamous cell neoplasms, glandular neoplasms, urachal carcinomas, Mullerian type tumors, NETs, melanocytic tumors, mesenchymal tumors, and haemato-lymphoid tumors are all recognized under the WHO classification of tumors of the urothelial tract.[5] The bulk of epithelial tumors are of the urothelial type, and approximately 95% of bladder tumors are of epithelial origin.[6] About 70% of cases of urothelial carcinoma present as non-muscle-invasive tumors (NMIBC), whereas the remaining cases present as muscularis propria-invasive disease (MIBC).[7] Additionally, within two years of receiving therapy for their invasive tumors, roughly 50% of patients who first presented with MIBC may relapse with metastatic sickess.[8]

The molecular features of MIBC and NMIBC are very different, and they arise through at least two different molecular pathways: NMIBCs develop through epithelial hyperplasia and the recruitment of branching vasculature. At the same time, MIBCs progress through either high-grade papillary neoplasms or urothelial CIS lesions.[9] Chromosome alterations, which typically involve tumor suppressor genes and proto-oncogenes that present the initial events leading to carcinogenesis (e.g., alterations in chromosome 9, 17, 13), loss of cell cycle regulation that is responsible for cellular proliferation, and invasion-metastasis are the three categories into which the molecular changes that occur in urothelial carcinoma of the bladder can be divided.[8] Anatomical tumor extent/staging, grade, nodal status, angio-lymphatic invasion, and other variables are traditional prognostic markers for bladder cancer.[7]

The behavior of the majority of bladder tumors cannot be predicted by conventional histological assessment of bladder cancer.[10] Additionally, because these conditions are molecularly diverse, it is improbable that a single marker will be able to accurately describe a tumor's potential and behavior, enabling trustworthy treatment decisions.[11] According to Birkham *et al*. and Giordano *et al*., the most dependable method for early detection of aggressive phenotypes and patient stratification for therapeutic decision-making is still the analysis of several markers.[12,13] Compared to a single marker, a combination of independent complementary markers yields a more appropriate outcome prediction.[14]

The total number of altered biomarkers had the highest predictive accuracy, according to a combined multimarker immunoexpression analysis that included tumor suppressors, cell cycle regulators, and proliferation markers.[13] The risk of bladder cancer progression increased incrementally in proportion to the number of altered markers.[14] Tumour suppressors, oncogenes, and proliferative markers are important, but so are epithelial to mesenchymal transition (EMT) markers like vimentin. This phenomenon has been connected to dangerous tumor biology, which leads to poor clinical outcomes in both MIBCs and NMIBCs, including decreased response to treatment, increased recurrence, poorer survival, and a propensity to metastasise.[15-19] Nuclear Ki-67 antigen expression is a measure of cell growth fraction and hence biological aggressiveness of a malignancy. Investigations established Ki-67 expression as an independent predictor of recurrence, progression, and response to therapy in patients with urothelial carcinoma of bladder.[20] The aim of the study was to observe the expression of Vimentin and Ki-67 in relation to histologic grade and muscle invasion in urothelial carcinoma of urinary bladder.

Methods

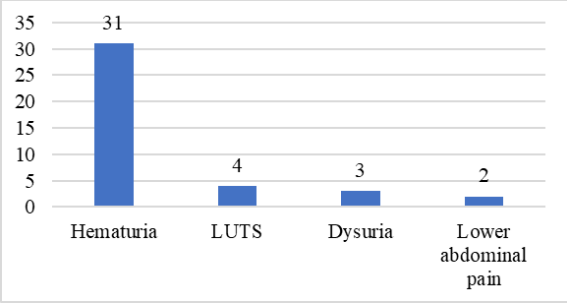
This cross-sectional study was carried out in Sir Salimullah Medical College, from March 2020 to February 2022. A total of 40 patients irrespective of age or sex, with histopathologically diagnosed urothelial carcinoma of bladder in the pathology department of Sir Salimullah Medical College, other teaching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

Results

Table 1: Distribution of patients by age (n=40)

Distribution of patients by age (n=40)		
Age group (in years)	**Frequency (n)**	**Percentage (%)**
30-40	1	2.5
41-50	3	7.5
51-60	15	37.5
61-70	13	32.5
71-80	8	20
Total	**40**	**100**
Mean± SD 60.8±9.8		
Distribution of patients by gender (n=40)		
Male	31	77.5
Female	9	22.5
Distribution of patients by smoking history (n=40)		
Smoker	24	60
Non-smoker	16	40
Total	**40**	**100**

Out of the 40 patients, 3 (7.5%) were from 41-50 years age group, 15 (37.5%) were from 51-60 years age group, 13 (32.5%) were from 61-70 years age group, and 8 (20.0%) were from 71-80 years age group. The mean age of the patients was 60.8±9.8 years (Table 1). Vast majority of patients (28; 70%) were in between 51 to 70 years of age. Among the 40 patients, 31 (77.5%) were male, while 9 (22.5%) were female (Figure 4.1). Male to female ratio was 3.4:1. Smoking history of the patients showed that 24 (60.0%) out of 40 were smoker, while 16 (40.0%) were non-smoker. Smoking duration of patients ranged from 10 to 28 pack-years. Smokers were all male patients. Some of the patients, smoker or non-smoker also had history of smokeless tobacco use with betel-nut for variable duration and amount.



* LUTS=Lower urinary tract symptoms

Figure 1: Distribution of patients by chief complaints (n=40).

31 out of 40 (77.5%) patients presented with the chief complaint of hematuria, while 4 presented with LUTS (urgency, frequency, nocturia), 3 with dysuria, and the remaining 2 with lower abdominal pain (Figure 1).

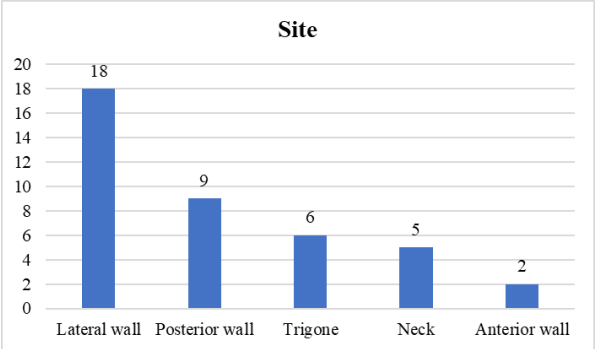


Figure 2: Distribution of cases by location (n=40)

Location of the tumors was recorded from cystoscopy report, and gross examination of samples. Out of the 40 cases, 18 (45.0%) were on lateral wall, 9 (22.5%) on posterior wall, 6 (15.0%) on trigone, 5 (12.5%) on neck, and the remaining 2 (5.0%) cases were on anterior wall (Figure 2).

Table 2: Distribution of cases by histologic grade (n=40)

Grade	Frequency (n)	Percentage (%)
High Grade	27	67.5
Low Grade	13	32.5
Total	**40**	**100**

Cases were graded according WHO/ISUP 2016 grading criteria (Epstein, 2016). Observation of the cases revealved that 27 out of 40 (67.5%), were high grade-carcinomas, while 13 (32.5%) were low-grade carcinomas (Table 2).

Table 3: Distribution of variants by histologic grade (n=18)

Variants	High Grade	Low Grade	Total
Squamous differentiation	7	1	8
Glandular differentiation	4	1	5
Nested variant	3	0	3
Sarcomatoid variant	2	0	2
Total	**16**	**2**	**18**

Among the 18 cases with divergent differentiation and variant forms, only 2 were low-grade, the remaining 16 (88%) were high-grade cases (Table 3).

Table 4: Distribution of cases by muscularis propria invasion (n=40)

Muscularis propria invasion	Frequency (n)	Percentage (%)
Present (MIBC)	25	62.5
Absent (NMIBC)	15	37.5
Total	**40**	**100**

Cases with unequivocal infiltration of malignant cells within bundles of muscularis propria were considered muscle invasive. 25 out of 40 cases (62.5%) had muscularis propria invasion, while 15 (37.5%) did not have muscularis propria invasion (Table 4). All of the variant forms were MIBCs (Table 4).

Table 5: Distribution of cases by grade and muscularis propria invasion (n=40)

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

Among the 25 MIBC cases 23 (92%) were high grade, while among the 27 high grade cases 23 (85%) were MIBC cases (Table 5)

Table 6: Distribution of cases by expression of Vimentin and Ki-67 (n=40)

Immunohistochemical markers	Frequency (n)	Percentage (%)
Vimentin		
Positive	28	70
Negative	12	30
Ki-67		
Positive	27	67.5
Negative	13	32.5

Immunostained slides were observed in 400x fields. 500 cells from each slide, within areas of highest intensity expression (hot spots), were analyzed. Vimentin was positive in 28 (70%) cases and Ki-67 was positive in 27 (67.5%) cases. (Table 6).

Table 7: Association between vimentin expression and histologic grade (n=40)

Grading	Vimentin			p value
	Positive	Negative	Total	
High grade	23 (85.2%)	4 (14.8%)	27 (100.0%)	**0.008**
Low grade	5 (38.5%)	8 (61.5%)	13 (100.0%)	

Among the 27 cases with high grade urothelial carcinomas, 23 (85.2%) were vimentin positive, while among the 13 cases with low grade urothelial carcinoma, 5 (38.5%) were vimentin positive. Fisher Exact test showed that high grade urothelial carcinoma had significantly more positive vimentin expression compared to low grade urothelial carcinoma (p=0.008) (Table 7).

Table 8: Association between vimentin expression and muscularis propria invasion (n=40)

Muscle Invasion		Vimentin		p value
	Positive	Negative	Total	
MIBC	23 (92.0%)	2 (8.0%)	25 (100.0%)	**<0.001**
NMIBC	5 (33.3%)	10 (66.7%)	15 (100.0%)	

Among the 25 cases with MIBCs, 23 (92.0%) were vimentin positive, while among the 15 cases with NMIBCs, 5 (33.3%) were vimentin positive. Fisher Exact test showed that cases with MIBCs had significantly more positive vimentin expression compared to cases with NMIBCs (p<0.001) (Table 8).

Table 9: Association between Ki-67 expression and histologic grade (n=40)

Grading	Ki-67			p value
	Positive	Negative	Total	
High grade	23 (85.2%)	4 (14.8%)	27 (100.0%)	0.001
Low grade	4 (30.8%)	9 (69.2%)	13 (100.0%)	

Among the 27 cases of high-grade urothelial carcinomas, 23 (85.2%) were Ki-67 positive, while among the 13 cases of low-grade urothelial carcinomas, 4 (30.8%) were Ki-67 positive (Table 9).

Table 10: Association between Ki-67 expression and muscularis propria invasion (n=40)

Muscle Invasion	Ki-67			p value
	Positive	Negative	Total	
MIBC	21 (84.0%)	4 (16.0%)	25 (100.0%)	0.006
NMIBC	6 (40.0%)	9 (60.0%)	15 (100.0%)	

Among the 25 cases of MIBCs, 21 (84.0%) were Ki-67 positive, while among the 15 cases of NMIBCs, 6 (40.0%) were Ki-67 positive. Fisher Exact test showed that there was significant statistical association between muscle invasive carcinomas and positive expression of Ki-67 (p=0.006) (Table 10).

Table 11: Correlation between vimentin and Ki-67 expression with histologic grade (n=40)

Marker	r_s	p value
Vimentin	0.506	0.001
Ki-67	0.265	0.098

*Spearman's correlation

Spearman's ranked correlation test showed that, there was significant moderate correlation between grading and vimentin expression, as r_s =0.506 (p=0.001). However, no significant correlation was present between grading and Ki-67 (r_s =0.265, p=0.098) expression (Table 11).

Table 12: Correlation between vimentin and Ki-67 expression with muscularis propria invasion (n=40)

Marker	r_s	p value
Vimentin	0.585	<0.001
Ki-67	0.324	0.041

*Spearman's correlation

Spearman's ranked correlation test showed that, there was significant moderate correlation between muscle invasive carcinomas (MIBC) and vimentin expression as r_s =0.585 (p<0.001). There was significant weak correlation between muscle invasive carcinomas (MIBC) and Ki-67 expression as r_s =0.324 (p=0.041) (Table 12).

Discussion

A total of 40 patients with urothelial carcinoma diagnosed histopathologically in the Pathology Departments of Sir Salimullah Medical College and Mitford Hospital, other teaching hospitals, and private institutions in Dhaka during March 2020 to February 2022, were included in this study. The present study findings were discussed and compared with previously published relevant studies. In the present study the mean age of the patients was found 60.8±9.8 years with a range from 37 to 78 years (Table 1). Maximum 15 (37.5%) patients were found in age group 51-60 years, followed by 13 (32.5%) patients in age group of 61-70 years which is very close to the age group 51-60 years. Previous studies conducted in Bangladesh and various south-asian countries also showed similar age distribution, with peak frequency of detection at 6th decade of life.[21-23] 31 (77.5%), out of the 40 patients were male and 9 (22.5%) were female. Therefore, male to female ratio was 3.4:1, which is concordant with Global Cancer Statistics 2018 (3.38:1).[24]

Among the participant cases of the study, 24 (60%) were smoker, and 16 (40%) were non-smoker. A study conducted by Chinnasamy et al. in India found that 71% of patients were smokers.[25] It is important to note that, various forms of smokeless tobacco may have effects on study population, which is difficult to ascertain, because of widely variable contents, and amount of tobacco use within study population Dwivedi et al.[26] Hematuria was the most common chief complaint of the study population (77.5%), followed by LUTS (urgency, frequency, nocturia), dysuria, and lower abdominal pain in 10%, 7.5%, and 2.5% respectively (Figure 1). Hematuria was also the most common symptom of bladder cancer found in other studies and is typically present in about 67-80% of patients.[27,28]

Other less common symptoms found in those aforementioned studies include: LUTS (frequency, urgency, etc.), abdominal pain, symptoms referable to UTI (burning/dysuria), obstruction, etc. Within

these study subjects, 18 had tumors arising on lateral wall (45%), followed by in descending order of frequency: 9 on posterior wall (22.5%), 6 on trigone (15%), 5 on neck (12.5%), and 2 on laterior wall (5%) (Figure 2). In a study performed on 4163 patient cohort, it was observed that, the most frequent tumor location was on lateral wall (39%), followed by posterior wall, trigone, dome, anterior wall, bladder neck, and ureteral orifice. In this study, tumors were graded according to "WHO Classification of Tumors of the Urinary System and Male Genital Organs, 2016" grading criteria into high-grade and low-grade carcinomas. It was observed that most of the cases (27 cases, 67.5%) had high-grade carcinomas, while, remaining 13 (32.5%) had low-grade carcinomas (Table 2). This observation is conflicting with the previously reported observations of many studies, that, approximately 70%-80% of all initially presenting carcinomas are low grade.[7] Unfortunately as many as 30% of low grade tumors-confined to superficial mucosa progress to a higher grade and/or stage of tumors and historically, up to 83% CIS patients developed invasive carcinoma if left untreated.[8]

In respective studies conducted in Bangladesh and India, Haque *et al*., Chinnasamy et al. reported high-grade tumors in 72%, 63.5%, 75.26%, and 55.5% of cases respectively, which is similar to our current study scenario.[21,25] Among the 18 cases with divergent differentiation and variant forms, only 2 were low-grade, the remaining 16 (88%) were high-grade cases (Table 3). Twenty five of the cases (62.5%) had MIBC, while 15 had NMIBC (37.5%) (Table 4). Among the MIBC cases 23 (92%) were high grade, on the contrary among the 27 high grade cases, 23 were muscle invasive (86%) (Table 5). Positive (novel) expression of vimentin was observed in 28 (70%) of the specimens and in respect of Ki-67 expression, 27 cases (67.5%) were Ki-67 positive, the remaining 13 cases (32.5%) were Ki-67 negative. (Table 6). In two respective studies on large cohort of cases Margulis *et al*. and Pfister *et al*. found positive expression in 48.2% and 72% of the cases respectively.[20,29]

Among the 27 cases with high grade urothelial carcinomas, 23 (85.2%) were vimentin positive, while among the 13 cases with low grade urothelial carcinoma, 5 (38.5%) were vimentin positive. Fisher Exact test showed that high grade urothelial carcinoma had significantly more positive vimentin expression compared to low grade urothelial carcinoma (p=0.008). (Table 7). Among the 25 cases with MIBCs, 23 (92.0%) were vimentin positive, while among the 15 cases with NMIBCs, 5 (33.3%) were vimentin positive. Fisher Exact test showed that cases with MIBCs had significantly more positive vimentin expression compared to cases with NMIBCs (p<0.001) (Table 8).

Among the 27 patients with high grade carcinomas, 23 (85.2%) were Ki-67 positive while among the 13 patients with low grade carcinomas, 4 (30.8%) were Ki-67 positive. (Table 9). Among the 25 patients with MIBC, 21 (84.0%) were Ki-67 positive while among the 15 patients without Muscle invasive bladder cancer, 6 (40.0%) were Ki-67 positive. (Table 10). In 2 studies conducted in Bangladesh Haque *et al.* (2018) and Chowdhury, (2024) both found significant association of Ki-67 with muscle invasiveness and/or grading.[21,30] In a series of 713, and 164 cases Margulis *et al*. and Quintero *et al.* observed that expression of Ki-67 is associated with higher grade, invasive carcinomas.[20,31] However, no significant correlation was present between grading and Ki-67 (r_s =0.265, p=0.098) expression. (Table 11). There was significant weak correlation between muscle invasive carcinomas (MIBC) and Ki-67 expression as r_s =0.324 (p=0.041) (Table 12). 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 has shown that although different markers have different expression patterns in regard to histologic grade and muscle invasion, overall Vimentin and Ki-67 expression has most statistically significant relationship with both grade and muscle invasion. Furthermore, total number of positive markers in patient cohort have statistically significant collaboration with prognostic parameters, namely histologic grade and muscle invasion.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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