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Original Article

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

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ABSTRACT

Introduction: *Bladder tumors are about six times more prevalent in developed countries than in developing ones (Kirkali et al., 2005). However, the incidence of bladder cancer is rising in developing countries like Bangladesh, with incidence, mortality, and 5-year prevalence rates of 1.1%, 0.84%, and 2.3%, respectively (GLOBOCAN, 2020). This study aimed to examine Vimentin expression concerning histologic grade and muscle invasion in urothelial carcinoma of the bladder.* ***Methods & Methods:*** *This prospective, cross-sectional observational 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.* ***Result:*** *In this study, 15 (37. 5%) cases were in age group 51-60 year, and 13 (32.5%) were in group 61-70 year, with the mean age at presentation 60.8±9.8 years, ranging from 37-78 years. 31 cases were male, with male to female ratio of 3.4:1. The predominant symptom was hematuria (77.5%). Most of the tumors (18; 45%) were located on lateral wall. The tumors were graded according to WHO grading criteria (2016). Regarding vimentin expression, it was seen that, both grade and muscle invasion were associated with positive vimentin expression (p=0.008 and p<0.001 respectively). Spearman's correlation test showed that vimentin expression has statistically significant correlation with both grade (rs=0.506; p=0.001) and muscle invasion (rs=0.585; p<0.001).*

Conclusion: *The outcome of this study has shown that positive vimentin expression has statistically significant relationship with both grade and muscle invasion.*

Keywords: *Vimentin, Urothelial Carcinoma, Grade, Muscle Invasion.*

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INTRODUCTION

An estimated 5, 49,000 new cases and 2, 00,000 fatalities occur from urinary bladder cancer each year, making it the tenth most frequent kind of cancer globally. Men are more likely than women to have it; the incidence and death rates for men are 9.6 and 3.2 per 1, 00,000 respectively, which is around four times higher than that of women worldwide [1]. According to Kirkali et al., bladder tumors are generally six times more common in industrialized nations than in underdeveloped ones [2]. Because of urbanization, industrialization, increased chemical usage, and increased tobacco use, urinary bladder cancer is becoming increasingly common in developing nations like Bangladesh [3]. In Bangladesh, bladder cancer incidence, death, and 5-year prevalence rates are 1.1%, 0.84%, and 2.3%, respectively.[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]. Up to 70% of patients who present with NMIBC (Stage pTa, pTis, or pT1), return, and about 25% of patients move to a higher grade and/or stage of the disease.[2] Additionally, within two years of receiving therapy for their invasive tumors, roughly 50% of patients who first presented with MIBC may relapse with metastatic illness [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

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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 represent the initial events leading to carcinogenesis (e.g., alterations in chromosome 9, 17, 13), loss of cell cycle regulation that accounts 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 reliably predicted by conventional histological examination of bladder cancer [10]. Epithelial to mesenchymal transition [EMT pathways] are exceedingly important in dictating aggressive behavior of tumors. This phenomenon has been linked to aggressive 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. Hence the detection of EMT markers, like Vimentin, plays a pivotal role in predicting tumor behavior and patient outcome [11-19]. The aim of the study was to observe the expression of Vimentin in relation to histologic grade and muscle invasion in urothelial carcinoma of urinary bladder.

METHODS & MATERIALS

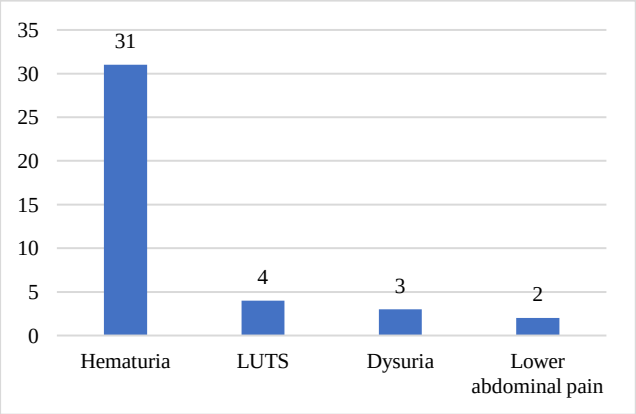
This cross-sectional study was carried out in Sir Salimullah Medical College, from March 2020 to February 2022. A total of 40 patients irrespective of age or sex, with histopathologically diagnosed urothelial carcinoma of bladder in the pathology department of Sir Salimullah Medical College, other teaching 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 – I: 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 I). 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).

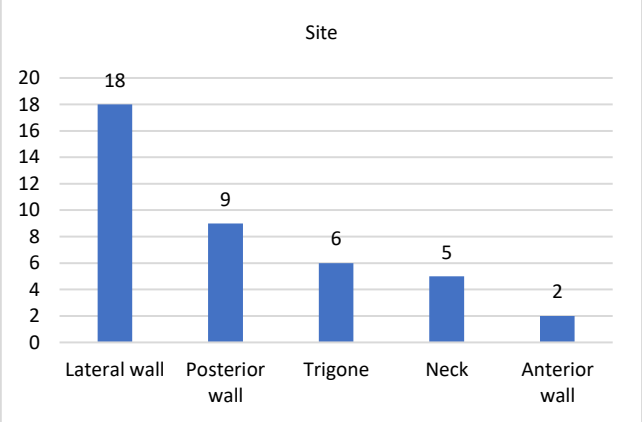


Figure – 2: Distribution of cases by location (*n*=40)

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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 – II: 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 revealed that 27 out of 40 (67.5%), were high grade-carcinomas, while 13 (32.5%) were low-grade carcinomas (Table II).

Table – III: 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 – IV: Distribution of cases by muscularis propria invasion (n=40)

Muscularispropria 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 IV).

Table – V: 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 V)

Table – VI: Distribution of cases by expression of vimentin (*n*=40)

Immunohistochemical marker	Frequency (n)	Percentage (%)
Vimentin		
Positive	28	70
Negative	12	30

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 (70.0%) cases. (Table VI)

Table – VII: Correlation between vimentin, with histologic grade (*n*=40)

Marker	r_s	p value
Vimentin	0.506	0.001

*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. (Table VII)

Table – VIII: Correlation between vimentin, and muscularis propria invasion (n=40)

Marker	r_s	p value
Vimentin	0.585	<0.001

*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). (Table VIII)

Table – IX: 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 IX).

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Table – X: 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 X)

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 I). 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 [20-22]. 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).[23] 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 [24]. 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.[25]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 [26,27]. 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 (Williams et al. 2004), 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 [20,24]. Among the 18 cases with divergent differentiation and variant forms, only 2 were low-grade, the remaining 16 (88%) were high-grade cases (Table III).Twenty five of the cases (62.5%) had MIBC, while 15 had NMIBC (37.5%) (Table IV). Among the MIBC cases 23 (92%) were high grade, on the contrary among the 27 high grade cases, 23 were muscle invasive (86%) (Table V). According to Epstein et al. (2016), about 90% of invasive tumors (≥pT1) are high grade. Positive (novel) expression of vimentin was observed in 28 (70%) of the specimens (Table 6) [28]. Rahmani*et al*. (2015), also noted vimentin expression in 69% of urothelial carcinomas in his study population [29]. 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). (Table VII) 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). (Table VIII) Among the 27 cases with high grade urothelial carcinoma, 23 (85.2%) were vimentin positive while among the 13 cases with low grade urothelial carcinoma, 5 (38.5%) were vimentin positive (Table IX). 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 IX). 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 X).

Limitations of the Study

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

CONCLUSION

The outcome of this study has shown that vimentin expression has statistically significant relationship with both histologic grade and muscle invasion.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

RECOMMENDATION

Vimentin may help to improve prognostic accuracy of urothelial carcinoma of urinary bladder. It may have a role in early detection of aggressive phenotypes. Large sample size, longer duration and multicenter studies would bring out more representative data. Follow-up of the patients' clinical outcome would be helpful for further evaluation.

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