Original Article

Expression of p53 and its correlation with grading of urothelial carcinoma of urinary bladder

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Abstract:

Background: *Urothelial bladder cancer (UBC) is a disease of significant morbidity and mortality. Staging and grading of these carcinomas are of utmost prognostic significance while strict segregation may be difficult and biological behavior of these tumors is highly variable. We evaluated the relationship of immune-histochemical expression of p53 protein with grading of urothelial carcinoma of urinary bladder.* ***Objective:*** *To determine the correlation between p53 protein expression and grading of urothelial carcinoma of urinary bladder.* ***Materials and Methods:*** *This cross-sectional study was conducted in the Department of Pathology and Department of Urology, Rajshahi Medical College & Hospital (RMCH) over the period of January 2018 to June 2019. It involved 50 cases of urothelial carcinomas diagnosed on trans-urethral resection specimens. Grading was done according to the WHO guidelines. Immuno-histochemical staining for p53 performed in all the cases, categorized as negative and positive expression taking 10% positivity as cutoff value, and correlated with the grade of urinary bladder carcinoma.* ***Results:*** *Majority (70%) of the patients were ≥ 60 years with a male to female ratio of 4.5:1. There were 17(34%) cases of low-grade and 33(66%) cases of high-grade urothelial carcinoma on histopathological examination. p53 expression was found positive in 24(72%) cases of high-grade carcinoma while only in 05(29%) cases of low-grade carcinoma. Significant difference in expression of p53 was found in different grade of urothelial carcinoma (P value <0.001).* ***Conclusion:*** *Our results corroborate with the opinion that in conjunction with histological grading, p53 immuno-marker may provide additional prognostic information in bladder urothelial carcinomas to stratify high risk patients.*

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Introduction

Bladder cancer is the commonest malignancy involving the urinary system, and the ninth commonest malignancy worldwide[1]. Over three-quarters (77%) of the tumors occur in men and smokers are four times more likely to develop urinary bladder cancer than non-smokers[2,3,4]. Incidence of bladder cancer increases with age[5], with its burden prevailed in developed countries until recently. However, because of increasing number of smokers and mass industrialization the paradigm is changing with developing countries bearing substantial burden of bladder cancer these days1. In South-East Asian region, the incidence and mortality due to bladder cancer are 1.8% and 1.3% respectively in both sexes[6]. In Bangladesh prevalence of urinary bladder carcinoma is 3.4% in men[7].

Most patients with bladder cancer are diagnosed after they present with gross or microscopic hematuria[8]. More than 90% of bladder tumors are urothelial carcinoma; 5% are squamous cell carcinomas; and 2% are adenocarcinomas[9]. The biological behavior of these tumors is highly

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variable[10]. Amongst all, grading and staging of bladder tumors are important prognostic factors[9,11,12]. Others include lymph node involvement, patient's age, vascular invasion, p53 overexpression, loss of E-cadherin[13,14]. Generally, low-grade carcinomas are associated with good prognosis compared to high grade carcinoma. But the outcome can be different in patients at the same pathological grading[15]. Also histopathological examination of urinary bladder carcinoma is subject to considerable intra and inter observer variation and separation between different grades can sometimes be very difficult especially in small biopsies which may show crushing and cautery artifacts and in cases where histologic features are borderline. Accurate prognosis with any single factor is difficult to predict. Several biological and molecular parameters have been suggested as potential prognostic markers for bladder cancer. Mutated TP53 gene is a common genetic abnormality in bladder urothelial carcinoma[14]. Numerous studies have evaluated association of abnormalities and mutations of p53 and prognosis in bladder cancers. To our knowledge, in Bangladesh, study of p53 protein expression in urothelial carcinoma has not been studied earlier. p53 protein expression profile along with histopathological grading can guide the oncologist to select high risk patients, and to initiate appropriate treatment modalities. Our study is designed to use p53 protein as an immunohistochemical biomarker to find out its relationship with different histological grades of bladder urothelial carcinoma.

Materials and Methods

This study was a descriptive cross-sectional study. The study was conducted in the Department of Pathology, Rajshahi Medical College, and Department of Urology, Rajshahi Medical College Hospital, Rajshahi over the period of January 2018 to June 2019. Patients aged between 20 years to 85 years admitted in the Department of Urology, RMCH or attended at outpatient department (OPD) of Urology between 01.01.2018 to 30.06.2019 with suspected bladder urothelial carcinoma were screened and confirmed cases were enrolled. Approval for the research protocol was obtained from the Ethical Review Committee, Rajshahi Medical College, Rajshahi prior to the commencement of the study (Ref. RMC/ERC/ 2017-201 9/ 119 Date: 25/ 11 /2018). Informed consent was sought for participant enrollment. Purposive convenience sampling technique was used.During the collection of specimens, patient information, demographic data, clinical findings, past medical history, risk factors and investigation findings were recorded systematically in a prepared proforma. All the cases were numbered chronologically and the same number was given to histological as well as in immunohistochemical slides. Tissue processing and staining were done according to standard protocol followed at Rajshahi Medical College. Sections were studied under light microscope to classify benign and malignant lesions and to select one representative paraffin block for immunohistochemical analysis. Grading of the tumor was done according to WHO/ISUP guidelines. Immunohistochemistry was done in Armed Forces Institute of Pathology (AFIP), Dhaka. Standard immunohistochemical method was applied for subsequent staining. Brown nuclear staining considered positive for p53. Expression of p53 was calculated as a percentage of labeled nuclei per 200 cells counted in most immunoreactive region of the tumor and categorized into negative and positive. Tumors with nuclear immunoreactivity of more than 10% were considered positive. Statistical analyses were carried out using SPSS version 20.0(SPSS Inc., Chicago, Illinois, USA). Descriptive analyses were performed. Chi-square tests were used to analyze categorical variables. Results having P-values <0.05 were considered statistically significant.

Inclusion criteria

Patients with following criteria were included in the study

- All patients with clinically suspected and later on histopathologically confirmed as having urothelial carcinoma.

Exclusion criteria

Patients with following characteristics were excluded.

- Patients clinically suspected of having bladder urothelial carcinoma but not confirmed by histopathology.
- Obtained biopsies that were inadequate and poorly preserved, and specimens showing evidence of marked inflammation were excluded.

Table I: Demographic characteristics of 50 patients confirmed with urothelial bladder carcinoma

Characteristics	N	%
Age (years)		
< 60	15	30
≥60	35	70
Mean ± SD (Min-Max)	63.03 ± 11.98 (25 - 84)	
Gender		
Male	41	82
Female	09	18
Socio-economic status		
Low	31	62
Middle	19	38
Personal history		
Smoking	36	72
Betel nut chewing	38	76
Total	50	100

Maximum (70%) patients were more than or equal to 60 years of age and 30% patients were below 60 years of age. Mean age (±SD) of the patients was 63.03 (±11.9) years. Male were predominant (82%). Maximum patients had the habit of smoking (72%) and betel nut chewing (76%).

Result

Majority (70%) of the patients were ≥ 60 years with a male to female ratio of 4.5:1. 17(34%) low-grade and 33(66%) high-grade urothelial carcinoma were diagnosed by histopathological examination. p53 expression was found positive in 24(72%) cases of high-grade carcinoma while only in 05(29%) cases of low-grade carcinoma. We observed no statistically significant difference between p53 immunoreactivities and age, sex or smoking habit ($P > 0.05$). Significant difference in expression of p53 was found in different grades of urothelial carcinoma (P value <0.05).

Table II: Clinical findings of 50 patients confirmed with urothelial bladder carcinoma

Clinical findings	N	%
Hematuria		
Present	48	96
Absent	02	04
Dysuria		
Present	27	54
Absent	23	46
Lower abdominal pain		
Present	28	56
Absent	22	44
Total	50	100

Most of the patients (96%) presented with hematuria. 54% had dysuria and 56% had lower abdominal pain.

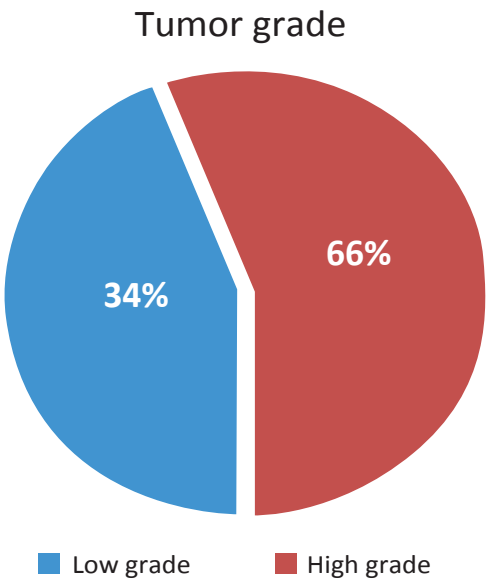

Figure 1: Tumor grade among 50 patients confirmed with urothelial bladder carcinoma by histopathology

According to WHO/ISUP histological grading, 33 (66%) were high grade and 17 (34%) were low-grade urothelial carcinoma.

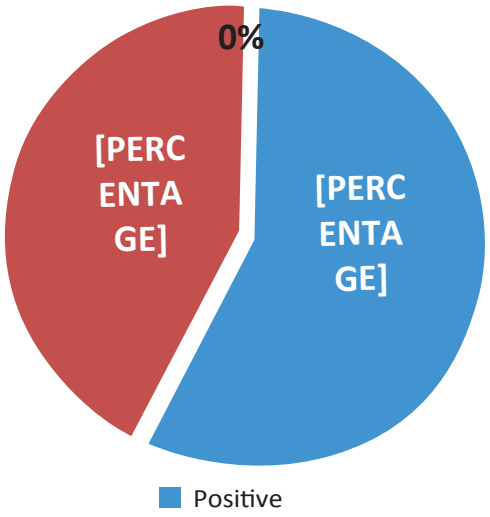

Figure 2: p53 expression among 50 patients with urothelial bladder carcinoma confirmed by histopathology

Among 50 patients, immunohistochemically 29 (58%) were p53 positive and 21 (42%) were p53 negative.

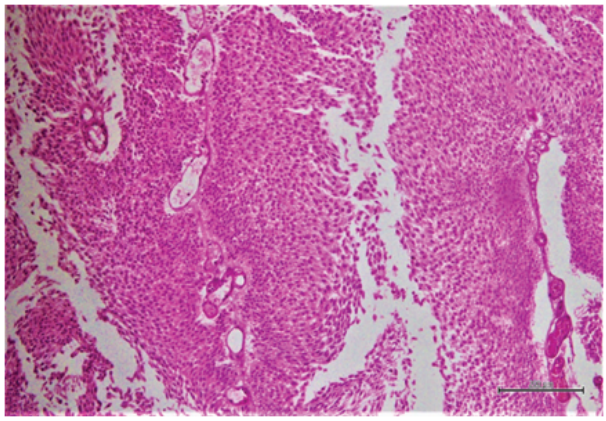

Figure 3: Photomicrograph showing low grade papillary urothelial carcinoma, (Case no. 06, H&E x100)

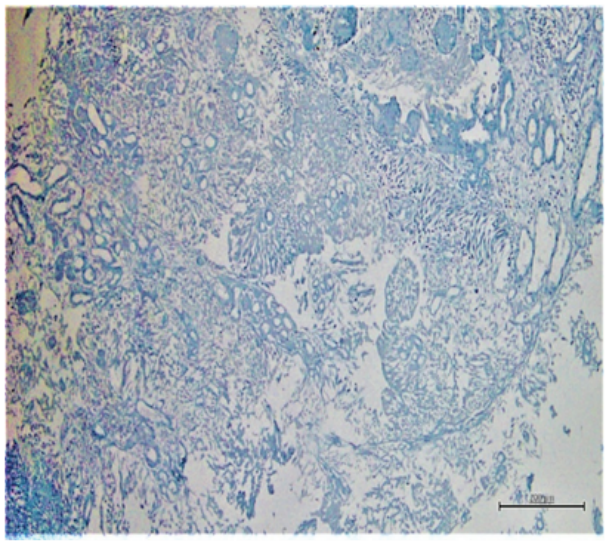

Figure 4: Photomicrograph showing negative p53 immunostaining in low grade papillary urothelial carcinoma (Case no. 06, H&E x100)

Table III: Relationship between tumor grade and p53 status among 50 patients confirmed with urothelial bladder carcinoma

Histopathology grade	p53 expression		Total n (%)	P
	Positive n (%)	Negative n (%)		
Low grade	12 (70.6)	05 (29.4)	17 (100)	
High grade	09 (27.3)	24 (72.7)	33 (100)	<0.05
Total	21 (42.0)	29 (58.0)	50 (100)	

Proportion of positive p53 expression was significantly higher among patients with high grade tumor (P < 0.05).

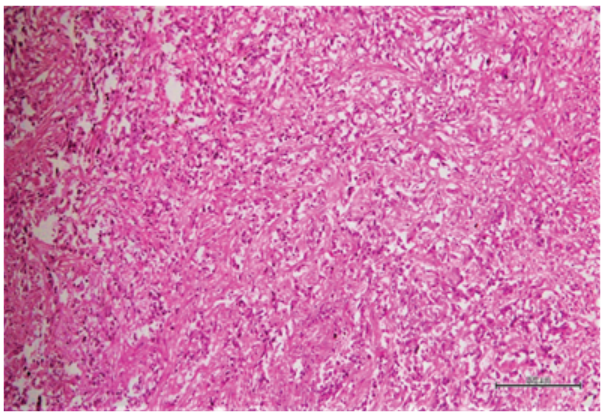

Figure 5: Photomicrograph showing high grade urothelial carcinoma, (Case no. 13, H&E x100)

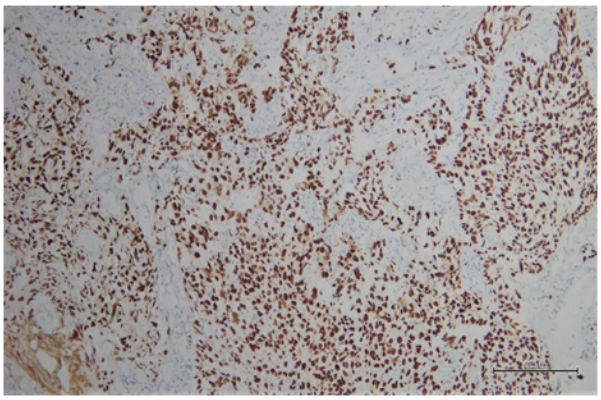

Figure 6: Photomicrograph showing positive p53 immunostaining in high grade urothelial carcinoma (Case no. 13, H&E x100)

Discussion:

We found the median age at presentation was 63 years (minimum 25 years, maximum 84 years) with male to female ratio 4.5:1. Kumar et al., (2017)[16] found mean age of the patients with neoplastic lesions was 55.6 years with male to female ratio 4:1, which is similar to our study. In their study majority of the bladder tumors occurred in the sixth and seventh decades (55.7 %.) which is in line with our study. In a study in 2012 Karam[17] found the mean age of the patients was 62.64 years with male and female ratio was 9:1, which was quite higher than our study. Gupta et al., (2009)[18] in Lucknow, India found mean age 60.2 ± 4.4 year but the male and female ratio was 8.8:1, which was higher than our study. There may be other confounding factors responsible for higher M: F ratio in the mentioned study.

Men had a threefold greater risk of developing bladder cancer than women[19]. The higher incidence of UBC in male may be due to the personal habit such as smoking and more exposed to toxic agents due to their occupation. In our study most of the male patients had habit of both cigarette smoking and betel nut chewing and females had habit of betel leaf with betel nuts; which is almost similar to a previous study by Chinnasamy et al., (2016)[20] that revealed most of bladder cancer patients (71.2%) had smoking habit. Chou et al., (2013)[21] found 24.9% of urothelial cancer patients had smoking habit; which is inconsistent with our result.

Our findings of almost all patients presented with microscopic hematuria and the relative proportions of other clinical features are in line with previous studies[9,20], except dysuria, which was almost five times higher in our study.

We found more high-grade (66%) than low-grade (34%) urothelial carcinoma which is almost similar to the study by Chou et al., (2013)[21]. In 2012 Karam[17] found 46% & 30% cases of high-grade & low-grade cases respectively. Rest of the cases were papilloma and PUNLMP. We found no PUNLMP cases in our study. In our country the probable cause of higher grade UBC may be low socioeconomic condition, lack of awareness, lack of urological treatment facilities as well as social and religious restrictions especially for female patients. Moreover, all our cases were from RMCH, which is a tertiary level hospital and so patients are admitted with advanced stage of disease or referred as complicated cases. In another study by Chinnasamy et al., (2016)[20] in India observed 64% high-grade and 36% low-grade carcinoma which were almost similar to our study.

We scored p53 expression according to the percentage of positive tumor cells. This simple method was easy to apply compared to complex scoring schemes that take into account combination of staining distribution (percent of positive cells), as well as staining intensity. All cases with 60–100% positive tumor cells demonstrated moderate-to-strong staining intensity, therefore, inclusion of staining intensity into immunohistochemical scoring scheme would not improve performance of the test at the chosen cut-off. In addition, evaluating intensity of staining is problematic as it is difficult to reproduce and it can vary with different protocols.

We observed no statistically significant difference between p53 immunoreactivities and age, sex or smoking habit ($P > 0.05$), which is quite similar to some previous studies[22,23,24]. Ozgoz et al., (2005)[25] found p53 overexpression in all of the three female patients; they reported that this might suggest that p53 mutation rates in urinary bladder cancers seen in females might be higher than those seen in males, but it was hard to say so, because of the limited number of female patients in the group.

In our study, proportion of positive p53 expression was significantly higher among patients with high grade tumor, ($P < 0.05$). There are numbers of studies in the literature conducted on p53 and tumor grade and different results have been obtained. Soini Y et al., (1993)[26] found more p53-positive cases in Grade II–III tumors than in Grade I tumors ($P = 0.004$), which is similar to our study. Esrig et al., (1994)[14] investigated alteration of p53 with both molecular analysis and immunohistochemistry and have shown that tumors with high p53 accumulation are high grade, stage and at high risk for recurrence. Their immunohistochemical results were similar to ours. Few studies reported that using the cell cycle proteins in combination might be useful for prognosis in bladder cancers. Hitchings et al., (2004)[27] assessed the prognostic value of p53 and p16 combined staining and alterations of both of them were more informative than staining p53 alone. Conversely some studies reject the predictive value of p53 mutation analysis and immunostaining for recurrence and progression[28,29,30].

The reason of the conflicting results in the literature about p53 expression may be due to a number of factors. The preferred antibody clone is one of them. DO-7 anti-p53 antibody was used in our study. Masters et al., (2003)30 assert that the DO-7 clone is less effective than the pAb1801 antibody. Interobserver inconsistency is one of the other reasons. Only strong nuclear staining was taken into account when scoring p53, weak and moderate intensity nuclear staining was ignored in the current study. Using the different cut-off points for p53 and heterogeneity of the series may lead to different results.

Conclusion

Some recent studies mention that p53 protein over-expression is a bad prognostic indicator for bladder urothelial carcinoma. In routine practice only grading and staging is done by histopathology, which cannot provide sufficient information regarding the aggressiveness of these tumors due to its highly unpredictable behavior. So, we performed this study with the aim to evaluate the association of p53 protein expression in various grades of urothelial bladder carcinoma. The findings of our study suggest that use of p53 immunomarker in conjunction with histopathological grading may provide significant prognostic information for stratifying high-risk patients in urinary bladder carcinoma.

Recommendations

Immunohistochemical expression profile of p53 protein in bladder urothelial carcinoma may be included as part of routine pathological evaluations. Larger sample size, longer duration, multicenter studies with reliable, reproducible, and equivalent standardized immunohistochemical techniques, use of other biomarkers, molecular

studies and follow-ups would bring out more representative data.

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