

ORIGINAL ARTICLE

p53 and Ki-67 Expression in Papillary Urothelial Carcinomas of Urinary Bladder, An Experience at Foundation University Medical College Islamabad, PakistanIram Kehkashan Khurshid¹, Mumtaz Ahmad², Sarah Javed³, Saba Aneeqa⁴, Atiya Gardezi⁵, Maryam Waqar⁶**ABSTRACT**

Objective: To determine the frequency of p53 and Ki-67 staining in cases of papillary urothelial carcinomas of urinary bladder and to correlate staining frequencies of p53 and ki 67 in low- and high-grade carcinomas.

Study Design: A cross sectional study.

Place and Duration of Study: The study was conducted in department of Histopathology, Foundation University Medical College and Hospital Rawalpindi for duration of six months, from 1st September 2017 to 28th February 2018.

Materials and Methods: Eighty-five patients of both genders undergoing trans urethral resection of bladder tumor were included in the study. The bladder biopsies were collected after TURBT and placed in 10% formal-saline for twenty-four hours. After tissue fixation, representative sections were taken and processed routinely for histology and immunohistochemistry at histopathology lab. Patients with histological diagnosis of low and high grade Papillary urothelial carcinoma were included in the study.

Results: Among 85 patients, 64 were females and 21 were males. The most frequent tumor was low grade papillary urothelial carcinoma seen in 44 patients while 41 patients had high grade carcinoma. Positive p53 expression was more in high grade carcinoma (32.94%) as compared to low grade carcinoma (27.1%) and negative p53 expression was more in low grade carcinoma (23.5%) as compared to high grade carcinoma (14.1%). However, a statistically non-significant p value of 0.132 was obtained on comparison of p53 expression with chi square test. Similarly, positive Ki-67 expression was seen in 45.9% of low-grade carcinomas and 37.65% of high-grade carcinomas and negative expression was seen in 9.4% of low-grade carcinomas and 7.06% of high-grade carcinomas which did not correlate with our histopathological diagnosis. p value calculated by chi square test for Ki-67 was 0.659 which is also non-significant in our study.

Conclusion: Our study concluded that p53 and Ki-67 immunostaining does not significantly correlate with histopathological grades of urothelial papillary carcinoma of urinary bladder in our local population. Therefore, immunohistochemistry expression of these markers cannot be solely relied upon for determining the grading of urothelial carcinomas.

Key Words: Immunohistochemistry, Ki-67, P53, Tumor Markers, Urinary Bladder Neoplasms.

Introduction

Bladder carcinoma is a 6th most frequent malignant neoplasm in the world with 7th amongst most

frequent malignancies in men and 17th in women.¹ The reported incidence of new bladder carcinoma cases is about 1 in 10 people before 75 years of age.² The most frequent tumor type of bladder carcinoma is Urothelial (transitional cell) carcinoma comprising 90% of all cases followed by squamous cell carcinoma and adenocarcinoma.^{3,4} Clinically, bladder carcinomas are divided into superficial non-muscle invasive tumors and invasive tumors.^{3,5,6,7} In non-muscle invasive carcinomas, mainstay of treatment is local resection while invasive tumors require cystectomy (Bladder removal) or additional adjuvant therapies.^{2,8} Early diagnosis of bladder carcinoma by pelvic ultrasound and cystoscopy (by urologist) helped in early treatment, reducing tumor morbidity.⁹ Urine cytology is also used for diagnosis

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as it is cost effective but less sensitive diagnostic modality.¹⁰ So coordinated efforts of urologists and the histopathologists for detection of lesion is essential for management of patients.¹¹ Apart from depth of invasion, the stage and grade of urothelial carcinoma has significant prognostic value.¹¹ Immunohistochemical expression of p53 and Ki-67 can be utilized for grading and staging of urothelial carcinomas^{12,13} and also as important prognostic markers.^{13,14}

A thorough search of literature showed that not much work is done on bladder carcinoma diagnosis with correlation of its histological grade and immunohistochemical markers expression in local Pakistani population.

This study was an effort to identify the histological grade of bladder tumor in small biopsies using histopathology and its correlation with immunohistochemical expression of p53 and Ki-67 among local Pakistani population which acted as a guide for clinicians in determining therapeutic management plan for patients.

Materials and Methods

After approval by the Ethical Committee, this comparative cross-sectional study was carried out. It was conducted at Department of Histopathology and Urology, Foundation University Medical College and Hospital, Rawalpindi. The study spanned 6 months duration from 1st September 2017 to 28th February 2018. The study population comprised eighty-five patients of both genders undergoing TURBT, selected by consecutive (nonprobability) sampling technique. Sample size was calculated using WHO sample size calculator. Patients with histological diagnosis of low and high grade Papillary urothelial carcinoma were included in the study. Patients with histological diagnosis of papillary urothelial carcinoma of low malignant potential and those receiving chemotherapy or radiotherapy prior to surgery and biopsies with insufficient material were excluded from the study.

The bladder biopsies were collected after TURBT and placed in 10% formal-saline for twenty-four hours. After tissue fixation, representative sections were taken and processed routinely for histology and immunohistochemistry at histopathology lab. An indirect IHC staining protocol was used consisting of four main steps: (1) Fixation – to keep everything in

its place, (2) Antigen retrieval – to increase availability of proteins for detection, (3) Blocking – to minimize pesky background signals and (4) Antibody labeling (Primary and secondary antibodies by DAKO) and visualization. The H&E-stained sections of bladder tissue was examined simultaneously by me and a senior consultant histopathologist under 10 and 40 objectives in the histopathology department, Foundation University Medical College, giving the exact histologic grade of the papillary urothelial tumor. The IHC slides of Ki-67 and p53 were also examined under 10 and 40 objectives by another consultant histopathologist. The low power examination i.e., 10 objectives helped to find the field with maximum positive cells. Then that area was examined under 40 objectives to calculate the cells with positive nuclear staining (brown to black) dividing it with the total number of cells in that area (positive and negative) and multiplying result with 100. Both markers give nuclear staining. p53 and Ki-67 were considered positive when more than 10% of the total tumor cells gave positive (brown) nuclear staining. The person who reported IHC slides was blinded to histopathological slide results and vice-versa. SPSS version 17 was used for data analysis including frequencies and percentages for age, gender, tumor grade (low, high), p53 and Ki-67 expression. Chi –square test was used to compare frequency of p53 and Ki-67 expression among low and high grade papillary urothelial carcinomas of the urinary bladder. p value of ≤ 0.05 was considered significant.

Results

A total of 85 patients were diagnosed with papillary urothelial carcinoma of bladder. Maximum number of patients 27(31.8%) were in the age range of 61-70years. 64 (75.3%) were female and 21(24.7%) were male, with a female to male ratio of 3:1. Among all patients 58 (68.24%) patients presented with bladder growth on cystoscopy followed by hematuria in 25 patients (29.41%) and flank pain in 2 patients (2.35%). On histological diagnosis 44 (51.8%) patients revealed low grade carcinoma and 41(48.2%) patients had high grade carcinoma. (Figure -1).

In this series, positive p53 expression was seen in 32.94% of high-grade carcinoma and 27.06% cases of low-grade carcinoma. Negative p53 expression was

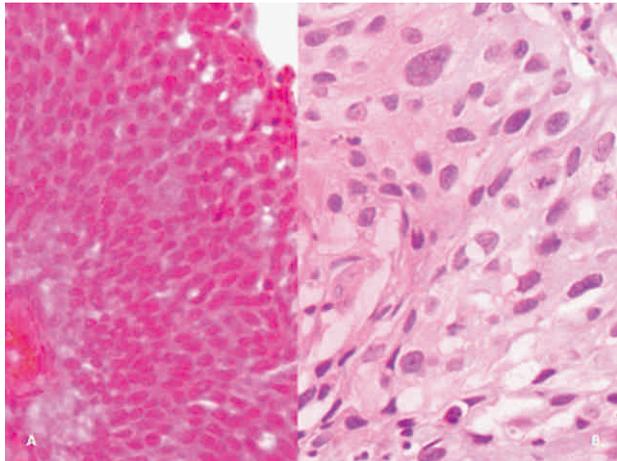


Fig 1: Relatively Bland Nuclear Features of Low-Grade Carcinoma (100X) A; As Compared To Pleomorphic Nuclei And Prominent Nucleoli in High-Grade Bladder Carcinoma (400 X) B H&E.

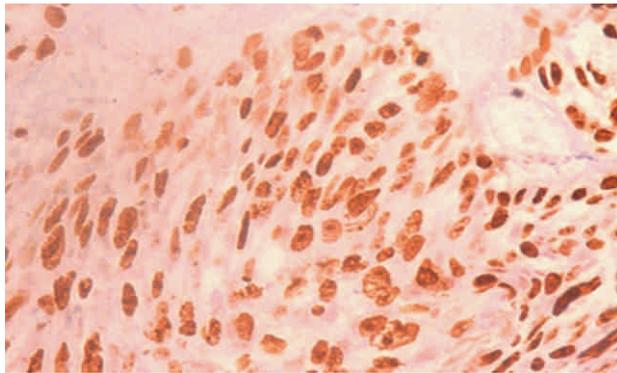


Fig 2: Positive Nuclear Staining of p53 (400X) IHC

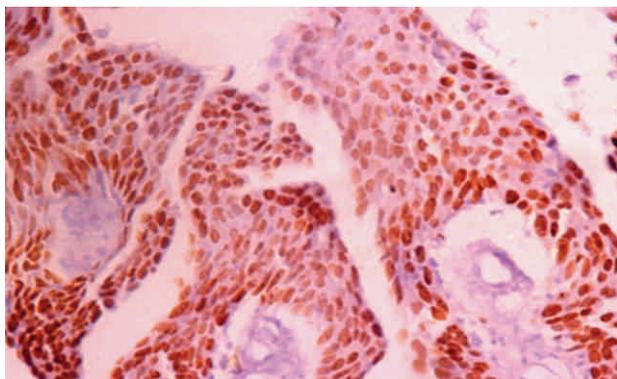


Fig 3: Positive Nuclear Staining for Ki-67 (200X) IHC

present in 23.5% of low-grade carcinoma and 14.1% cases of high-grade carcinoma. On comparing histopathological diagnosis with p53 expression by Chi square test the p value was non-significant p=0.132. Positive Ki-67 expression was seen in 45.9% of low-grade carcinomas and 37.65% of high-grade carcinomas and negative expression was seen in 9.4% of low-grade carcinomas and 7.06% of high-

Table I. Frequency of P53 and KI-67 Expression among Low- and High-Grade Urothelial Neoplasms

p53 Expression	Number of cases N=85	Frequency %	Low grade	High grade
Positive	51	60.0	23(27.06%)	28(32.94%)
Negative	34	40.0	21(24.71%)	13(15.29%)
				p value 0.132
KI-67 Expression	Number of cases N=85	Frequency %	Low grade	High grade
Positive	71	83.5	36(45.88%)	35(37.65%)
Negative	14	16.5	8(9.41%)	6(7.06%)
				p value 0.659

grade carcinomas which did not correlate with the histopathological diagnosis. p value calculated by chi square test for Ki-67 was 0.659 which is proved non-significant.

Discussion

Urinary bladder carcinoma is amongst the most frequently occurring carcinomas in Pakistan causing significant morbidity and mortality. For these reasons, patient's diagnostic workup is very important as it guides the clinicians about the treatment modalities. Immunohistochemistry is an adjunctive tool to confirm the histopathological grade of bladder carcinoma.¹² Mutations in p53 and Ki-67 are among the key contributors in carcinogenesis of bladder cancers.¹³ In the current study, the urothelial carcinoma was more frequent in females (75.3%) however worldwide bladder carcinoma is more frequent in males. This discrepancy may partly be due to the fact that female patient's turnover was more in our hospital. A research done by Rukhsana Parveen Samo et al in 2019 states higher incidence of bladder carcinoma in males (61.4%) as compared to females (38.6%).¹⁵ In their study, more subjects had high grade bladder carcinoma (65.10%) with highest number of patients in age range of 46-60 years¹⁵ while in our study, low grade carcinoma was more frequent and highest number of patients were in 61-70 years age bracket. Another study done in 2014 by Telli showed results comparable to our study, exhibiting low grade bladder carcinoma to be more frequent bladder tumor.¹⁶ A study done by Rafal stec et al., in 2019 on 134 patients showed positive correlation of p53 and

Ki-67 with tumor grade unlike our study.¹⁷ Mirja geelvink et al., in 2018 took higher percentage of tumor cells to be positive, i.e Ki-67 >15% was taken as positive for high grade bladder carcinomas.¹² A study done by Dipti Gajjar et al in 2019 On 314 patients of bladder pathologies revealed Immunohistochemistry requirement for diagnosis in 48 cases. Out of those 48 cases, 16 cases were of low-grade bladder carcinomas exhibiting p53 expression of >10% in 15 cases (93.75%) and Ki-67 of 15-40% in all 16 cases (100%)¹⁸ unlike our study.

A study by Stanislav Ziaran et al., in 2020 on p53, Ki-67 expression in bladder carcinomas took p53>10% and Ki-67>15% as positive. Ki-67 showed positive correlation with high grade morphology (unlike our study) as well as predictor of tumor recurrence while p53 did not prove to be significantly correlated with tumor progression or recurrence.¹⁹

A Brazilian study conducted on 43 patients of transitional cell carcinoma of urinary bladder revealed 20 patients of high grade while 23 patients with of low-grade carcinomas. The Ki-67 positivity was observed in total of 25 patients (58.1%), out of which 18 patients were of high-grade carcinomas (41.9%), while 7 patients were of low-grade carcinomas (16.2%). The Ki-67 was negative in 18 patients (41.9%), out of which 2 patients were of high-grade carcinomas (4.7%) and 16 patients were of low-grade carcinomas (37.2%). The expression of p53 was observed in total of 11 patients (25.6%), all of which were having high grade carcinomas, while expression was negative in 32 patients (74.4%) out of which 9 patients were of carcinomas (20.9%) and 23 patients were of low-grade carcinomas (53.5%).²⁰

A study by Thakur et al., in 2017 showed majority male patients (88.2%) with only 11.8% females. Cases reported as low grade were less (44.5%) as compared to high grade (55.5%). High expression of p53 and Ki-67 were seen in high grade carcinomas with statistically significant p value, contrary to our study.⁴

Conclusion

This study concluded that p53 and Ki-67 immunostaining is not significantly correlated with histopathological grades of urothelial papillary carcinoma of urinary bladder in our local population. Therefore, immunohistochemistry expression of these markers cannot be solely relied upon for

determining the grading of urothelial carcinomas.

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