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Original Article

Immunohistochemical Expression of P53 In Low-Grade and High-Grade Papillary Urothelial Carcinoma of the Bladder

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ABSTRACT

Introduction: Bladder cancer is the fourth most common genitourinary malignancy with urothelial carcinomas comprising 90% of all primary bladder carcinomas.

The protein TP53 is a nuclear phosphoprotein that acts as a tumor suppressor gene. Being a regulator of cell proliferation and pro-apoptotic gene, a mutation in p53 can nullify its normal functions and increased expression of the mutant protein is regarded as a predictor of poor prognosis of urothelial tumors.

Objective: To compare and determine the immunohistochemical expression of p53 gene overexpression in low and high-grade Papillary urothelial carcinoma.

Material and Methods: This cross-sectional study was done in the Histopathology section of the Department of Pathology, Pakistan Institute of Medical Sciences from March 2014 to April 2015. It included 65 cases of urothelial carcinomas. Immunohistochemical expression of p53 was performed according to standard protocols and correlated with age, sex, grade, and depth of invasion(stage).

Results: Out of the total 65 cases, 22 (33.2%) low-grade and 43 (66.2%) high-grade urothelial carcinoma cases were included in the study. Positive expression of p53 was seen in 41 (95.3%) cases of high-grade, and 12 (54.5%) cases of low-grade tumors.

Conclusion: The evaluation of p53 nuclear overexpression combined with histological grade and pathological stage may give more accurate information about the biological behavior of urothelial carcinoma of the urinary bladder.

Keywords: Urothelial carcinoma bladder, Low-grade urothelial carcinoma, High-grade urothelial carcinoma, p53 nuclear overexpression

Introduction

Urothelial carcinoma (UC) or Transitional cell carcinoma comprises 90% of all primary tumors of the urinary bladder and it is the most common tumor encountered by urologists. In the United States of America, it is the fourth most common cancer in men after prostate, lung, and colorectal cancer[1]. Other relatively less common malignancies of the urinary bladder include Squamous cell carcinoma and Adenocarcinoma. Urothelial cancer can also develop in the lining of the renal pelvis, ureter, prostate, and urethra [2]. In Pakistan bladder carcinoma is one of the top ten malignancies in men and the most common urological malignancy in both sexes.

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A study done in Karachi showed that out of 69% of the bladder lesions, 93% were Urothelial Carcinoma [3].In recent years, significant advances have been made to unveil the molecular mechanisms of carcinogenesis in the urinary bladder.[4,5] Two different molecular pathways are considered to be involved [6].

Low-grade papillary carcinoma (LGPC) is associated with a mutation in the FGFR3 or in some cases mutations in RAS genes. On the other hand, high-grade in situ/muscle-invasive carcinoma is characterized by the alteration of p53 and RB genes. Functional loss of these key genes, which play an important role in the control of the cell cycle, leads to the accumulation of additional mutations and deletions of genes resulting in an aggressive phenotype. A thorough understanding of the molecular nature of urothelial cancer is hoped to help in early diagnosis and develop new modalities for managing and treating these tumors [6,7,8]. Currently, the diagnosis of patients with invasive or noninvasive

Urothelial Carcinoma and their treatment is primarily based on the tumor grade and stage. However, according to many studies, tumor grade and stage alone have limited ability to predict tumor progression.[9]

Many tumor markers (Ki67, p53, RB, p21, and cyclin D1)[6,10] have been studied for their potential use in the assessment of the prognosis of Urothelial Carcinoma. However, a recent international consensus panel on prognostic markers for bladder cancer emphasized that certain markers such as p53, appear promising in predicting the progression of bladder cancer[11].In bladder cancer, a number of studies have shown a high correlation between p53 gene mutation and its nuclear accumulation can be detected by immunohistochemistry (IHC) and mutation detection by DNA sequencing [12]. A significant advantage of IHC over sequencing is that this technique can be easily performed in routine pathology laboratories. Immunohistochemical analysis of p53 overexpression is commonly used as a prognostic marker for mutational analysis.

The rationale of this study is to study the frequency of p53 overexpression in low-grade and high-grade urothelial carcinoma in our population and to assess the relationship of p53 with different clinical and pathological parameters such as age, sex, types of papillary urothelial carcinomas, tumor grade, and stage.

Materials and Methods

This cross-sectional study was conducted in the Pathology Department of the Pakistan Institute of Medical Sciences Islamabad. It took One year from 1-03-2014 to 4-04-2015. The sampling technique used was Non-probability consecutive sampling. The minimum sample size calculated was 65. It was calculated by using the WHO sample size calculator with the following: [12]

Confidence interval	95%
Absolute precision required	95%
Anticipated population proportion	58.6%
Sample Size	65

All biopsy specimens obtained through transurethral resection of bladder tumor (TURBT) and radical cystectomy of any age and sex with the morphology of invasive papillary urothelial carcinoma will be selected for p53 immunohistochemical staining. Only those tumors showing the morphology of papillary urothelial carcinoma will be selected for p53 immunohistochemical staining. The sampling does not include other types of bladder cancer.

After approval from the hospital ethical committee 65 paraffin-embedded blocks of papillary urothelial carcinoma bladder specimens were selected and immunohistochemically stained for overexpression of p53 during a one-year period. The grade of tumors (low or high grade) was determined using the classification proposed in 1998 by the world health organization and the international society of Urological Pathology (WHO/ISUP system) [5].

Immunohistochemical staining was performed using the avidin–biotin complex technique on three to five-micron slices of paraffin sections. For each case, a single representative slide was selected for staining and histologic evaluation. Briefly, slides were deparaffinized and rehydrated into water. Slides underwent antigen retrieval in Citrate buffer solution (Cat.No 00-5000) in the water bath at 95 ^{0}C for 40 minutes. Staining of p53 was performed using a monoclonal antibody (Leica DO-70). The extent of nuclear reactivity was classified into two categories (Positive and negative). No nuclear reactivity or few positive cells (1-10% tumor cells) was considered negative, while heterogeneous nuclear reactivity (10-50 % tumor cells) and homogenous intense nuclear staining (50 -100% cells) were considered positive. The percentage of positive staining was calculated in 100-300 tumor cell nuclei. Quotients (positive tumor cells/total counted tumor cells) calculated as percentages were rounded to the nearest integer. The frequency of p53 staining in low and high-grade urothelial carcinoma is evaluated in terms of both positive /negative nuclear staining for p53 [12].

The data was assessed in SPSS version 21. Mean and standard deviation (SD) was calculated for quantitative data like age. Frequency and percentage were presented for qualitative data like gender, the intensity of p53 nuclear staining, and grades (low and high) of urothelial carcinoma. The Chi-square test is applied to calculate the frequency of p53 overexpression between low and high grades of urothelial carcinoma. A p-value of ≤ 0.05 will be significant.

Results:

Out of a total of 65 study cases, 4 cases (6.15%) were obtained through radical cystectomy. The rest of the 61 cases (93.8%) were obtained through transurethral resection of bladder tumor (TURBT). Age distribution of the cases showed that 59 (90.8%) of the patients

were more than forty years of age and six (9.2%) patients were less than forty years old. The age range of the patients was between 20 to 95 years. The mean age of the patients was 61.60 ± 14.46 years. Out of 65 study cases, 63 patients were males (96.9%) and only two patients (3.1%) were females. Male to female ratio is 31:1.Forty-three (66.2%) cases were high-grade urothelial carcinoma of the bladder (UCB) and 22 (33.2%) cases were of low-grade UCB, respectively.

High-grade papillary urothelial carcinoma was the most commonly diagnosed tumor on histopathology. Among high-grade urothelial carcinoma, 41(95.3%) cases showed p53 overexpression while 2 (4.7%) cases showed no expression of p53 immunostaining. Out of 22 cases of low-grade urothelial carcinoma 12 (54.5%) cases were positively stained for p53 immunostaining while 10 (45.5%) cases were negative for p53 Immunostaining respectively. Out of 43 positive high-grade urothelial carcinoma cases 32(74.4%) cases showed >50% cell exhibiting nuclear staining for p53 immunostain while 10(23.3%) cases showed 10-50% positivity. One case showed 1(2.3%) <10% staining and no case was completely negative.

Out of 22 low-grade urothelial carcinoma cases, 3 (13.6%) cases have 50-100% nuclear p53 staining while 8(31.8%) cases showed staining of 10-50% cells, 7(31.8%) cases showed <10% nuclear staining and 4 (18.2%) cases were completely negative for p53 immunostain as shown in table 1.

In the present study, p53 staining was also evaluated in terms of the stage of the tumor as shown in Figures 1 and 2. All High Grade Urothelial Carcinoma of the Bladder were pT1 at least and most of the pT2 and pT3 cases were also HGUCB. There was no significant difference between males and females in rates of positive staining for nuclear p53 protein.

Table 1: Frequency of P53 Nuclear Staining in High-Grade and Low Grade Papillary urothelial carcinoma

Histological grade	P53 nuclear staining		P value <.05
	Positive	Negative	
High Grade	14	2	0.00006
Low Grade	12	10	

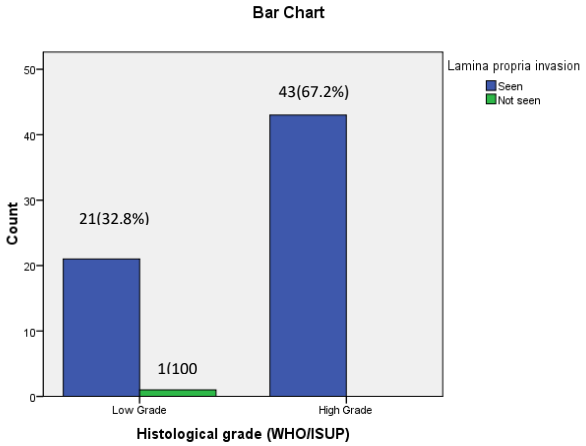

Figure 1: Frequency distribution of p53 expression with the stage (pT1) of Papillary Urothelial carcinoma of the bladder (n=65)

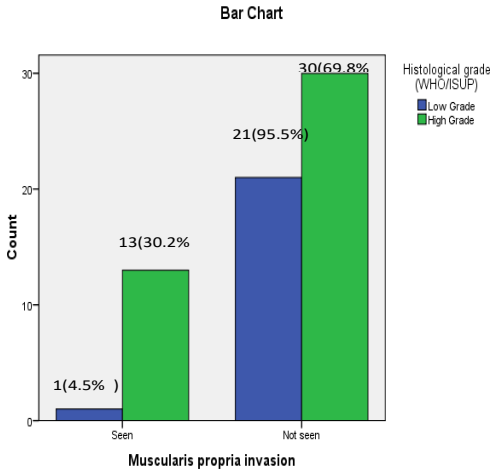

Figure 2: Frequency distribution of grade with the stage (pT2) of Papillary Urothelial carcinoma of the bladder (n=65).

Discussion

Urothelial carcinoma is a recurrent neoplasm that progresses to an infiltrating, very aggressive disease in a significant number of cases. In this study, we studied the frequency of p53 overexpression in low and high-grade urothelial carcinoma. The present study has shown a high correlation between p53 nuclear accumulation and high-grade urothelial carcinoma as detected by immunohistochemical methods so it may also be important to assess mutations of the p53 gene directly as a prognostic indicator in bladder cancer. The parameters for patients in this study matched those in the literature, mostly men (88%) with a median age of 62 years [9]. This might be due to hormonal differences or tobacco smoking, a main risk

factor for bladder cancer. Another Pakistani study also showed male predominance (79.8%), with a median age of 59.1 years in males and 58.8 years in females [13].64 cases of both low and high-grade urothelial carcinoma showed lamina propria invasion and 53 of them were positive for p53 overexpression while 11 cases were negative for p53 which were mostly low-grade tumors. 1 case neither showed lamina propria invasion nor muscularis propria invasion (pTa). So no statistically significant relationship can be assessed in terms of p53 staining in noninvasive and invasive urothelial carcinoma. This study showed a strong association between the intensity of staining with tumor invasiveness and grade. Almost all HGPUC cases were positive for p53 protein expression except for two cases. A statistically highly significant relationship was observed for pT1 and pT2 regarding p53 staining percentages; the staining percentage of pT1 below 10% and the staining percentage of pT2 by 10% or over were determined significantly high. In consistency with the study of Mumtaz et al[14], the rates of positive staining for nuclear p53 protein in the present study were higher in males, while in contrast to this Halemi et al study showed increased p53 expression in females as compared to males. Lin et al.[15] have reported p53 gene mutation in 33/54 males and 14/21 females, although the correlation was not studied. Another study conducted by Toktas in Turkey, correlated nuclear p53 accumulation with prognosis, they include a total of 90 patients with urothelial carcinomas, using an old version of the WHO classification and found that those tumors which expressed p53 had a higher rate of recurrence and progression and shorter survival[16].

In another study by Desai S et al, the clinical significance of p53 and Her-2/neu expression was evaluated [17] .They included 67 patients with invasive bladder cancer who have gone through radical surgery. The positive staining for p53 and Her-2/neu expression was seen in 44.8% and 39% of patients. The expression of both markers was significantly associated with higher tumor grades and pathologic stage along with the lymph node status. The patients showing greater co-expression of both markers had the worse prognosis. Similarly, Yildiz et al. used dual cocktail immunostain for p53 and cytokeratin 20 for the diagnosis of nonneoplastic and neoplastic bladder biopsies. The positivity for both of these markers was mainly seen in carcinoma in situ and carcinomas while cases showing reactive atypia or mild dysplastic changes were negative or showed focal staining mainly in the superficial urothelium [16].In this study the co-relation of P53 expression is also evaluated with muscularis propria invasion which is one of the prime prognostic factors in urothelial malignancies. Although 13 out of 14 cases that were muscle invasive exhibited expression of p53, this marker cannot be used as a surrogate marker for muscle invasion as 70% of non-muscle invasive urothelial carcinomas also showed expression of this marker.

The results reported here are consistent with the idea that p53 mutations are related to bladder cancer progression.

Conclusion

As a large percentage of bladder tumors are under staged, also separation of low and high-grade tumors can sometimes be very difficult, especially in small biopsies which may show crushing and cautery artifacts. Therefore analysis of p53 gene mutations could be useful as an adjunctive tool in grading urothelial carcinomas.

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