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Exploring the Role of BK Polyomavirus and SV40 T Antigen in Urothelial Cell Carcinoma of the Bladder

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Abstract

Background: *Urothelial carcinoma (UC) of the bladder is the 7th most common malignancy. BK Virus (BKV) has been associated with oncogenicity; however, the association between Urothelial Carcinoma and BKV remains inconclusive.* ***Objectives:*** *To investigate whether BK polyomavirus contributes to oncogenesis in immunocompetent hosts and clarify the prognostic significance of SV40 T antigen expression on human bladder cancer by SV40 T-antigen immunostaining. To evaluate the immunohistochemical expression of p53 nuclear reactivity using anti-p53 monoclonal antibodies in urothelial cell carcinoma of the bladder and to correlate the immunohistochemical expression with other clinicopathological parameters such as age, gender and tumor grade.* ***Materials and methods:*** *Sixty-six cystoscopic biopsies of Urothelial Carcinoma (UC) were immunohistochemically stained by antibody against SV40 T-antigen and anti- p53.* ***Results:*** *45.5% of the tumors were high grade. Muscle invasion was seen in 45.5% of the tumors. SV40 T antigen was not detected in all samples. p53 was expressed in 27.3 % of tumors. p53 expression was significantly correlated with tumor grade and muscle invasion. Tumor recurrence was significantly associated with p53 expression and tumor grade.* ***Conclusion:*** *BKV has no causal relation with UC in immunocompetent patients. p53 expression is a poor prognostic factor for UC. It showed a significant statistical. correlation with higher tumor grade, muscle invasion, and higher recurrence rate.*

Keywords: *Urothelial Carcinoma (UC), SV40, Large T-antigen (TAg), p53, BK Polyomavirus (BKV), Immunohistochemistry.*

1. Introduction

Bladder cancer is a significant global health issue, with approximately 573,278 new cases and 212,536 deaths reported in 2020.Incidence and mortality rates vary across regions, particularly in countries with a very high Human Development Index [1]. Urothelial carcinoma is the predominant type of bladder cancer in high-income countries, characterized by heterogeneity that necessitates risk stratification**.[2]**

This type of carcinoma has been linked to polyomaviruses, which can cause clinical illnesses, especially in immunosuppressed individuals [3, 4]. One specific Polyomavirus of interest is Simian Virus 40 (SV40). SV40 is a DNA virus believed to suppress the tumor-suppressing p53 protein's transcriptional properties in humans through its SV40 large T-antigen and SV40 small T-antigen. Large T-antigen is thought to play a significant role in neoplastic processes by deregulating p53, a critical protein responsible for regulating cell death (apoptosis) and cell cycle arrest in response to cellular damage**.[5]**

Numerous studies, including animal research, have reported an association between PV infection and the development of human bladder cancer**.[6]**

However, the role of PV in oncogenesis in individuals with genitourinary cancer who have a functioning immune system is still a subject of controversy and has not been definitively established. Initially, some studies suggested that BK virus (BKV), a type of polyomavirus, might play a role in the pathogenesis of bladder urothelial carcinoma**.[9-7]**

Given the potential implications of polyomaviruses and their interaction with p53 in oncogenesis, the main objective of the current study is to investigate the association between BKV and urothelial carcinoma in individuals with intact immune systems (immunocompetent patients). The study involves analysing bladder cancer cystoscopic specimens using advanced immunohistochemical methods to assess the expressions of SV40 large T-antigen (TAg) and p53 protein. The study's specific objectives include evaluating p53 nuclear reactivity in urothelial cell carcinoma (UCC) of the bladder through the application of anti-p53 monoclonal antibodies and establishing correlations with various clinicopathological parameters. Ultimately, the research aims to gain profound insights into the involvement of Polyomavirus and p53 in bladder cancer progression, potentially guiding future therapeutic strategies and prognosis evaluation.

2. Materials and Methods

This retrospective study included sixty-six cystoscopic biopsies of Urothelial carcinomas of the urinary bladder collected from Cairo University and Private labs in 2019-2020. Other types of neoplasms were excluded from the study. The tissue samples were processed routinely, fixed in 10% buffered formalin, dehydrated, and embedded in paraffin. Sections of 3-4 µm thickness were cut from the formalin-fixed, paraffin-embedded tumor blocks. Three sections were prepared from each tissue paraffin block: one for Hematoxylin and Eosin (H&E) staining for histopathological reassessment, one for immunohistochemical staining by SV40, and the last one for immunohistochemical staining by p53.

Immunohistochemical Staining for SV40: For the SV40 immunostaining, four-micron-thick sections were cut from the formalin-fixed, paraffin-embedded tissue blocks. The sections were deparaffinized, rehydrated, and subjected to antigen retrieval by boiling in citrate buffer (pH 6.0) in a microwave oven. To block endogenous peroxide activity, the slides were treated with 4% hydrogen peroxide in methanol. Biotin-labeled secondary antibody was applied, followed by peroxidase-conjugated streptavidin. The sections were stained with diaminobenzidine, counterstained with hematoxylin, and then mounted. Positive nuclear reaction to PV TAg was defined as positive staining, and renal allograft tissues with positive BK virus infection served as positive controls. Figure.1

Immunohistochemical Staining for p53: The same steps as described for SV40 immunostaining were followed for p53 immunostaining. Anti-p53 mouse monoclonal antibody was used, and breast carcinoma cells were used as positive controls. Samples with at least 10% nuclear reactivity were considered positive for p53. The nuclear overexpression of p53 detected through immunohistochemistry indicates a mutation in the P53 tumor suppressor gene.

Statistical Analysis: Data was entered and analyzed using the Statistical Package of Social Science Software program (SPSS), version 25.[10] Quantitative variables were presented using mean and standard deviation, while qualitative variables were presented using frequency and percentage. Comparison between groups for qualitative variables was performed using Chi-square or Fisher's exact tests, and for quantitative variables, the independent sample t-test was used. Time to event analysis using Kaplan Meier technique was employed to demonstrate the cumulative recurrence rate over time/weeks, and subgroup analysis was performed using Log rank test. A significance level of $p \leq 0.05$ was considered statistically significant.

3. Results and Discussion

The age of the patients ranged from 23 to 85 years old, with a mean age of 59.4 ± 13.9 SD. Half of the patients (33 cases) were 60 years old or less, and the other half were above 60 years. The majority of cases (90.9%) were males, with a male to female ratio of 9.98:1. Regarding tumor characteristics, 54.5% of tumors were low-grade, while 45.5% were high-grade. Additionally, 54.5% of tumors were non-invasive, and 45.5% were invasive. Recurrence occurred in 12 out of 66 cases (18.2%). All studied cases showed negative SV40 T-antigen immunostaining, while p53 immunostaining was positive in 18 cases (27.3%) and negative in 48 cases (72.7%). Figure (2,3).

Table (1):

Variable	Description	N (%)
Grade	Low	36 (54.5%)
	High	30 (45.5%)
Tumor Invasiveness	Invasive	30 (45.5%)
	Non-invasive	36 (54.5%)
Recurrence	Yes	12 (18.2%)
	No	54 (81.8%)
Anti-SV40 IHC	+VE	0 (0%)
	-VE	66 (100%)
p53	+VE	18 (27.3%)
	-VE	48 (72.7%)

The statistical analysis revealed no significant relationship between p53 expression and age and gender. However, p53 immunohistochemical expression was significantly correlated with the grade of tumor, tumor invasion, and recurrence ($p< 0.0001$) Figure 1,2,3. The cumulative recurrence rate was also significantly correlated with p53 expression, grade of tumor, and invasion ($p<0.0001$, 0.011, and 0.011, respectively). Age did not affect the cumulative recurrence rate ($p=0.837$).

Table (2):

Variable	P53 +VE (n=18)	P53 -VE (n=48)	*p*- value
Age Range	60.9 ± 12.2 (37 - 84)	58.8 ± 14.6 (23 - 85)	0.580
Age	6 (33.3%)	27 (56.3%)	0.097
	12 (66.7%)	21 (43.8%)	
Gender	18 (100%)	42 (87.5%)	0.178
	0 (0%)	6 (12.5%)	
Grade	0 (0%)	36 (75%)	0.000
	18 (100%)	12 (25%)	
Invasiveness	18 (100%)	12 (25%)	0.000
	0 (0%)	36 (75%)	
Recurrence	10 (55.6%)	2 (4.2%)	0.000
	8 (44.4%)	46 (95.8%)	

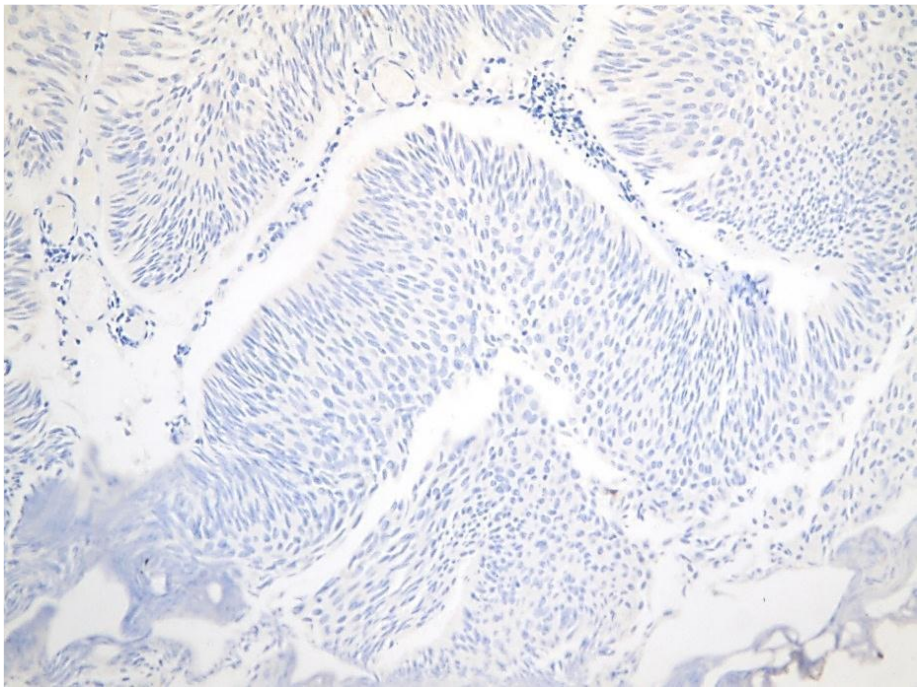

Figure (1): Immunohistochemical analysis of the PV TAg protein, using monoclonal antibody to SV40 TAg, in urothelial cell carcinoma of the bladder showing negative staining (IHCx4).

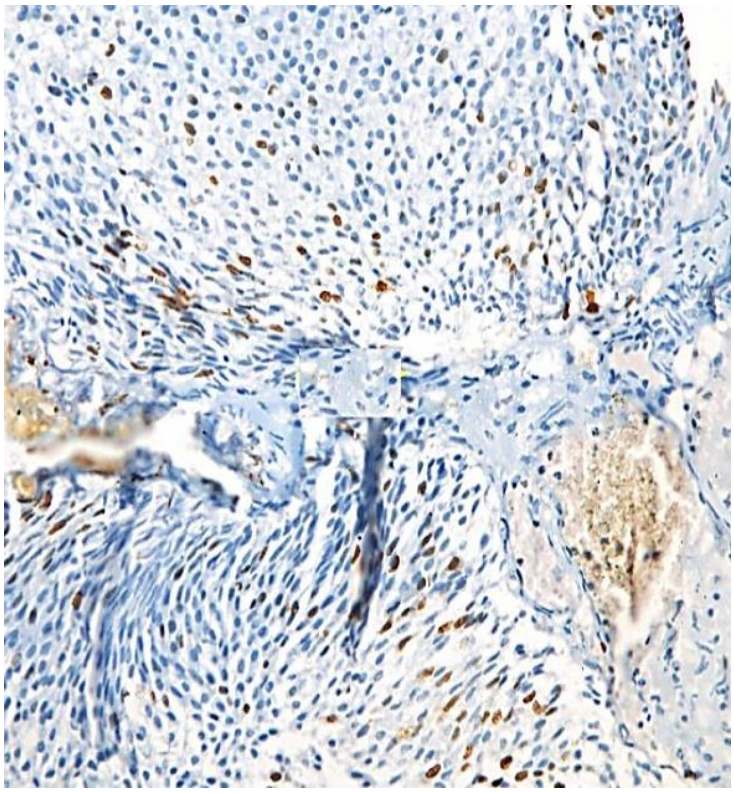

Figure (2): Immunohistochemical staining showed positive nuclear staining of p53 in a high-grade tumor, score +2. (IHCx20).

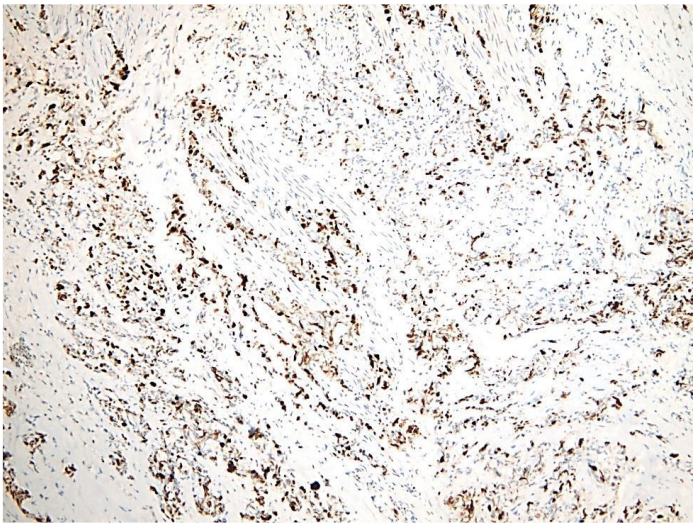

Figure (3): Immunohistochemical staining showed high-grade Urothelial Carcinoma showing positive nuclear staining of p53, score +3 (IHCx10).

Bladder cancer is the 10th most common cancer worldwide, with an estimated 573,278 new cases in 2020, representing 3% of all cancers.[1].

The major prognostic factors in bladder carcinoma are the depth of invasion into the bladder wall and the degree of tumor differentiation, which refers to the tumor's stage and grade [11, 12]. In the present study, 45.5% of the tumors examined were found to be high-grade .

We examined cases of bladder carcinoma and found that 45.5% of them showed muscle invasion, while 54.5% were free of invasion. Similar findings were reported [13], which also showed a considerable proportion of non-muscle invasive cases (70% and 67.8%, respectively). However, the results differed from a study [14], in which a higher percentage (75%) of cases showed muscle invasion. These varying rates of muscle invasion in bladder carcinoma cases suggest that different factors or patient populations may influence the outcomes across studies .

The role of BKV in urothelial carcinoma in immunocompetent individuals remains uncertain, and the divergent findings in various studies may be attributed to different detection methods and study populations. Subsequent research, however, presented conflicting results. Other studies demonstrated that BKV DNA was found in both neoplastic (cancerous) and normal bladder mucosa with equal frequency, which did not support the involvement of BKV in urothelial carcinoma in immunocompetent individuals.[15]

There were studies that supported the association of BKV with urothelial carcinoma in immunocompetent individuals, using urine cytology and polymerase chain reaction (PCR) for detection [16-18]. On the other hand, some studies did not find evidence supporting the association of BKV with urothelial carcinoma in immunocompetent individuals, using immunohistochemistry (IHC), chromogenic in situ hybridization (CISH), quantitative PCR (QPCR), and urine samples for DNA testing.[21-19 ,15]

This study explores the molecular interactions between Large T antigen (TAg), an oncoprotein of polyomavirus, and two key proteins involved in cell cycle regulation: p53 and Retinoblastoma family (pRB) proteins. The interaction between Large TAg and p53 family protein results in the inhibition or inactivation of p53, leading to disturbances in the cell cycle's S-phase onset. As a consequence, cells surpass the cell cycle checkpoint, accumulating genetic changes during each cell cycle [22]. Similarly, the interaction between Large TAg and pRB causes the release of E2 factors of transcription factors, prompting dormant cells to enter the S-phase of the cell cycle.[23]

In 2013 a study [24] proposed that dysplastic effects resulting from the inactivation of p53 or pRB in BKV-infected urothelium may play a crucial role in the development of cancer in the bladder urothelium. This concept aligns with the idea of multiple carcinogenesis cascades.

The present study, using immunohistochemistry, did not detect Polyomavirus (PV) large T antigen (TAg) in all examined samples. This finding is consistent with several other studies, such as Rollison et al. (2007), which also failed to find evidence of BK Polyomavirus infection in bladder tumor samples or surrounding mucosa[20].

The nuclear overexpression of p53 detected through immunohistochemistry indicates a mutation in the P53 tumor suppressor gene, which is a common occurrence in neoplastic urothelium [25]. Studies have shown that alterations in the tumor suppressor p53 play a significant role in the multistep process of tumorigenesis in various human cancers.[26]

In this study, 27.3% of the cases (18 out of 66 cases) exhibited positive p53 overexpression. Soini et al. in 1993 detected mutations of the p53 gene and positive immunohistochemical expression for the p53 protein in 40% to 60% of urothelial carcinomas in their studies[27].

The relatively lower proportion of positive p53 expression in the current study can be explained by the fact that, in some tumors, the p53 gene protein product does not accumulate in the nucleus despite the presence of P53 gene mutations [28]. This suggests that there might be alternative mechanisms affecting p53 protein expression in a subset of cases, leading to its absence in the nucleus despite gene mutations.

In this study, the expression of p53 showed no statistically significant correlation with either the age or gender of the patients. The p-values for age and gender were 0.097 and 0.178, respectively, indicating that there was no significant relationship between p53 expression and these demographic factors (Table 2). Similarly, a study in 2004 also could not identify any statistically significant correlation between p53 expression and the age or gender of the patients[29].

In summary, the expression of p53 in this study and other related studies did not appear to be influenced by age or gender, suggesting that these demographic factors may not play a significant role in determining p53 expression levels in the context of the investigated urothelial carcinoma.

In this study, a significant direct correlation was observed between p53 expression and the tumor grade in bladder cancer cases, indicating that higher p53 expression levels were associated with higher tumor grades. This finding is consistent with meta-analysis by Liao [30] which also reported a significant positive association between p53 expression and tumor grade .

In this study, a significant correlation was observed between p53 immunohistochemistry and the cumulative recurrence rate, indicating that p53 expression was significantly associated with tumor recurrence (p-value=0.00) (Table 2). Consistent with the findings of this study, previous research in 2017 [31] reported p53 expression as a poor prognostic factor for tumor recurrence

Furthermore, the cumulative recurrence rate was found to be significantly correlated with the tumor grade in this study (Table 1). Similar results were reported in analyses by Stavropoulos et al[32] .

4. Conclusion

In summary, the study suggests that BK Polyomavirus (BKV) infection is not associated with urothelial carcinoma in individuals with a healthy immune system, but it should be thoroughly investigated in immunocompromised patients. P53 emerges as a potential unfavorable prognostic factor in Urothelial Carcinoma (UC), indicating the need to customize treatment approaches accordingly. Additionally, P53 shows promise as a predictive marker for recurrence in cases of urothelial carcinoma, offering valuable insights for clinical management.

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