# Polyomavirus Hominis 1(BK virus) Infection in Prostatic Tissues: Cancer versus Hyperplasia

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**Purpose:** Polyoma virus hominis 1, better known as BK virus (BKV) infection might be a predisposing factor for prostate cancer (PCa). The aim of this study was to compare the frequency of BK virus infection in pathological specimens of patients with PCa compared to patients with benign prostatic hyperplasia.

**Materials and Methods:** From July 2011 to June 2012, paraffin-embedded tissue blocks of patients with PCa (60 specimens) and also with benign prostatic hyperplasia (60 specimens) were investigated. After DNA purification, existence of virus nucleic acid was assessed by polymerase chain reaction.

**Results:** Viral DNA was identified in 9 patients (15%) with BPH and 17 patients (28%) with PCa ($P$ =.076). In patients with PCa, viral DNA was observed more often in those with lower total Gleason scores ($P$ = .045).

**Conclusion:** The frequency of BK virus infection in PCa patients was higher than BPH patients. BK virus was more often observed in patients with lower Gleason scores. Less detection of BK virus DNA in overt cancer may prove the activity of the virus which paves the way for tumorigenic transformation at early stages of PCa.

**Keywords:** BK virus; isolation; purification; polyomavirus infections; complications; prostate; virology; prostatic neoplasms; tumor virus infections.

## INTRODUCTION

Polyomavirus hominis 1, better known as BK virus (BKV), is a polyomavirus which is widely spread in human populations. Sero-epidemiological studies have confirmed the existence of BK virus antibodies in 60% to 80% of adults in the world.[1] Therefore, BK virus can persist in the body and create asubclinical latent infection which can be activated in immune suppressed individuals and rarely in healthy people.[2]

BK virus genome contains three different regions:[1] early coding region which codes the major tumor antigen (t-Ag), minor tumor antigen (t-Ag), and truncated tumor antigen (truncated T-Ag) which has been discovered recently;[2] late coding region which codes viral proteins of the capsid (VP1, VP2, and VP3) and also agoprotein; and[3] the non-coding control region containing necessary initiation and regulation elements for the transcription of the two previous regions.[3,4]

Carcinogenic feature of BK virus is highly dependent on early coding region which codes a nuclear phosphoprotein of 97 kDa named Tag. This protein is homologue ofSimian virus 40 (SV40) Tumor Ag(TAg) based on various biological and biochemical properties. SV40 TAg is an adenosine triphosphatase (ATPase) with helicase activity which binds to viral and cellular DNA to activate their replication and is bound to products of p53 and retinoblastoma (RB) tumor-suppressing genes and deactivates their functions. Current evidences demonstrate that the expression of TAg and SV40 BK virus alters the integrity and stability of the host cellular genome and contributes to structural and numerical aberrations in the chromosome.[2]

BK virus is known to produce persistent infection in kidney and ureter. Therefore, BK virus-related urothelialcancers are highly probable.[5] Among all urogenital tumors, prostate cancer (PCa) is an important cause of mortality in men.[4] This cancer is the most prevalent malignancy in men and is the second cause of cancer deaths in aged men in the USA.[6,7] In a study by Hossieni and colleagues, the prevalence of PCa in Iranians older than 40 yearsold was 3.4%.[8]

The aim of this study was to investigate the relationship of BK virus infection with PCa by comparing the frequency of BK virus DNA in cancerous and non-cancerous pathological prostate specimens.

## MATERIALS AND METHODS

From July 2011 to June 2012, paraffin-embedded tissue blocks of patients with PCa (60 specimens) and benign prostatic hyperplasia (BPH) (60 specimens) who were operated in our center were evaluated. Patients with PCa were chosen as case study and those with BPH as control group.All these patients were diagnosed at our center in Tehran and were over 50 years old. The

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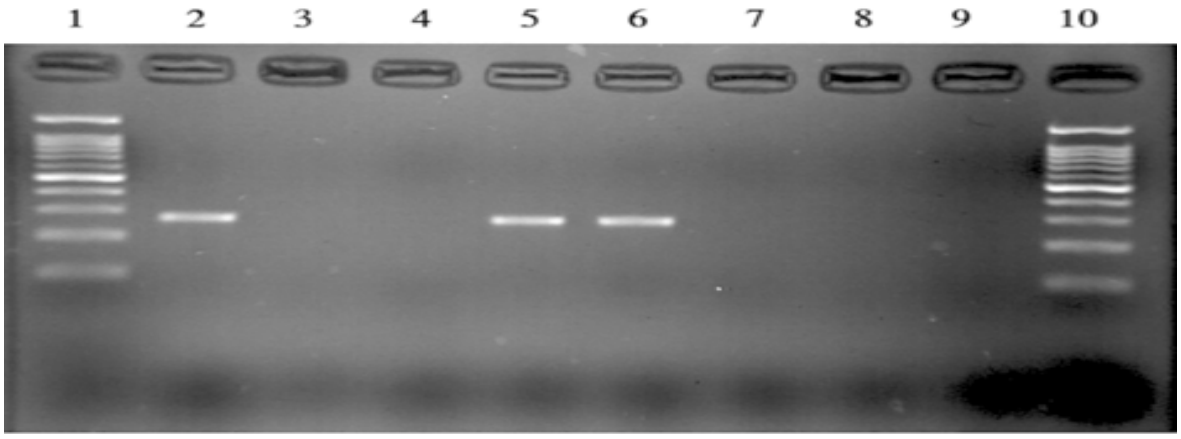


**Figure.** Polymerase chain reaction products of BK partial genome derived from prostate cancer paraffin embedded tissues. 1) DNA marker (100 base pair); 2) positive control; 3) negative control; 4) negative sample; 5,6) positive sample; 7,8,9) negative sample; 10) DNA marker.

diagnosis of PC aand BPH was confirmed by an experienced uro-pathologist. Six 5-10 µm sections were obtained from paraffin-embedded blocks of tumor site. QIA amp-DNA formalin-fixed, paraffin-embedded (FFPE) tissue extraction kit (Qiagen, Dusseldorf, Germany) was used to extract and purify BK viral DNA of prepared sections. After DNA extraction in specimens, presence of beta-globin DNA was evaluated by polymerase chain reaction (PCR) method and specific primers (GH20 and PC04) based on Saiki and colleagues report.[9] Specific primers (VP1-327-1 and VP1-327-2r) and PCR conditions for BK virus genes were derived from Jin protocol.[10] Accu Power PCR PreMix (Bioneer, Seoul, South Korea) which consisted of Top DNA polymerase (1 U), dNTP (250 µM), Tris-HCl (pH 9.0) (10 mM), KCl 30 (10 mM), MgCl2 (1.5 mM) with template DNA (100 - 200 ng) and primers (10 pmol) were used for PCR reactions. Also, thermal cycling with the following conditions, five minutes of denaturation at 94°C, followed by 35 rounds of an amplification cycle consisting of 35 seconds denaturation at 94°C, one minute annealing at 50°C for beta-globin and 55°C for BK virus and oneminute extension at 72°C, and a final extension cycle of fourminutes at 72°C, was used.

### *Statistical Analysis*

Two separate positive BK virus specimens from the urine of infected patients were used as positive controls. The Statistical Package for the Social Science (SPSS Inc, Chicago, Illinois, USA) version 17.0 was used for data analysis. Nominal and ordinal variables were compared by chi square and Mann-Whitney tests, respectively. Independent samples *t*-test was employed to compare continuous variables.

## RESULTS

In this study the mean age of patients with prostate hyperplasia was 68 ± 8.9 years old (age range of 50 to 95 years old). Also, the mean age of patients with PCa was 67.14 ± 9.96 years old (age range of 40 to 95 years old). Gleason score of tumor was categorized from 5 to 10. The highest score was 10, which was observed in 4.2% of the patients. Characteristics of this score in patients with PCa are summarized in **Table**.

### *PCR for Beta-Globin Gene in Paraffin Blocks*

After DNA extraction in specimens, presence of beta-globin DNA was evaluated by PCR method with 368 bp PCR product. This test was considered as a control for next molecular steps, and results of all assessed specimens were positive. Positive results demonstrated successful DNA extraction.

### *PCR for BK Virus in Paraffin Blocks*

After performing PCR for BK virus, in which a 287 bp segment was expected, of all 120 tissue specimens, 26 (21.7%) specimens were positive for BK virus DNA. From 60 PCa tissue specimens, 17 specimens (28.3%) and from 60 studied BPH tissue specimens, 9 specimens (15%) were positive for BK virus DNA. There was no significant relationship between the presence of BK virus DNA and the age of patients ($P$ = .38)

### *BK Virus and PCa*

There was no significant relationship between the presence of BK virus DNA and PCa of patients ($P$ = .076). Odds ratio rates of PCa were 2.24 (0.9–5.5), measured according to presence of BK virus in studied patients. These rates in patients with PCa and those with BPH were 1.5 (0.8–2.7) and 0.7 (0.4–0.9), respectively ($P$ = .076). However, there was a significant relationship between the presence of BK virus DNA and Gleason score ($P$ = .045). So Gleason scores were categorized into PCa risk categories. Gleason scores less than seven, seven and more than seven were grouped into low, moderate, and high risk categories, respectively. BK virus was discovered in eight (53%), five (25%) and four (16%) patients with low, moderate and high risk PCa, respectively ($P$ = .047).

However, there was a significant relationship between the presence of BK virus DNA and Gleason score (spearman $r$ = -0.26, $P$ = .045) (**Figure**).

## DISCUSSION

In this study,BK virus DNA was observed in 28% of PCa patients and 15% of BPH patients. These infection rates are of the highest reported rates. Lauand colleagues studied 30 PCa specimens. They stated that insitu hybridization test demonstrated the presence of BK virus in only two specimens (7%).[1]Also, in a study

**Table.** Characteristics of studied patients.

| Groups | Values |
|---|---|
| **BPH** | |
| Number of patients | 60 |
| Age, (mean ± SD) years | 68.0 ± 8.9 |
| **PCa** | |
| Number of patients | 60 |
| Age, (mean ± SD) years | 67.1 ± 10.0 |
| **Gleason score, No. (%)** | |
| 5 | 2 (3) |
| 6 | 13 (22) |
| 7 | 20 (33) |
| 8 | 9 (15) |
| 9 | 11 (18) |
| 10 | 5 (8) |

**Abbreviations:** BPH, benign prostatic hyperplasia; PCa, prostate cancer; SD, standard deviation.

by Akgüland colleagues in 2012, the presence of BK virus was verified in only 1 of 85 paraffin-embedded PCa tissues.[11] In 2008, May and colleagues revealed that none of their 213 PCa specimens had BK virus DNA.[12] They reported that BK virus lacked any significant role in cancer pathogenesis.

Higher frequencies of infection have been reported by few other researchers. Balis and colleagues reported the presence of BK virus in 19% of fresh frozen prostate tissues of 42 patients with PCa.[13] Russo and colleagues reported BK virus infection in 85% of specimens from PCa patients.[14]A main reason for discrepancies between these studies might be due to various assessment methods for BK virus in PCa specimens, since paraffin-embedded tissues enable fewer virus extractions compared to fresh frozen specimens. In other words, fresh frozen specimens contribute to more repeatable results and have higher sensitivity.[15] Furthermore, various molecular methods have been usedin these studies. Some studies have used nested PCR to extract more viral DNA.[16] Several studieshave suggested prostate as a suitable environment for the growth of infectious agents such as carcinogenic virus DNA and pointed to potential role of these viruses (e.g. John Cunningham (JC) virus) in incidence of PCa.[15] They claim that BK virus has a role in progression of PCa has been supported by other studies assessing the presence of viral TAg and/or p53 gene mutations. For instance, immunohistochemistry analysis in a study demonstrated the position of TAg and p53 in cytoplasm of cancer cells while in those cells negative for TAg, p53 was located in the nucleus. This emphasized the role of BK virus as a co-factor in pathogenesis of PCa. It suggested the role of BK virusin disruption of a cellular path way which may lead to cell cancer.[14]

Also, another study revealed that p53 was nuclear in negative TAg cells.[17] Furthermore, these cells had wild-type and mutant p53 genes. P53 in cells with TAg were always cytoplasmic and locating these two proteins in the cytoplasm was not associated with the presence of mutations in their nuclear sequence.[18] Considering these results together, researchers have suggested that wild-type p53 can be deactivated by TAg in the cytoplasm. In addition, several studies have revealed that despite locating TAg in carcinogenic cell cytoplasm, lack of viral VP1 expression in such cells implies lack of viral replication.

It is not easy to understand the mechanisms underlying viral oncogenic activities and the methods they employ to organize tumor microenvironment.[19-21] The “hit and run hypothesis” seems the most valid theory to justify a co-factorial role of BK virus in PCa onset and progression.[17] This hypothesis also helps to explain why BK virus is present in cancerous cells but is less present at the protein expression stage.[22] The ability of polyomavirus to interfere with the cell cycle could induce the infected cells to reach the critical point of no return during oncogenic transformation.[23]A meta-analysis on a total of 1106 cancer cases, ranging from 7 to 328, and 1068 control cases, ranging from 11 to 385, showed that the prevalence of BK virus was significantly higher in cancer tissues than in control tissues ($P < .0001$).[22] In the current study BK virus was observed in a higher frequency of PCa patients compared to BPH patients. BK virus DNA was detectedin 28% of patients with PCa which is almost twice the rate of infection in BPH patients which was 15%.

In our study, a significant relationship was found between low Gleason scores and BK virus in cancerous specimens ($P = .045$). Das and colleagues also noted the role of BK virus in early stages of PCa, but failed to detect the presence of BK virus in advanced stages of cancer.[6] Lower detection of the BK virus DNA in overt cancerous cells may point to the activity of the virus which paves the way for tumorigenic transformation at early stages of PCa. Viral fitness in the tumor cells is no longer needed to charge the tumor causality to the virus itself.[22] However, Russo and colleagues have reported a significant relationship between higher Gleason scores and higher BK virus DNA.They also reported a significant relationship between higher Gleason scores and rates of mutant p53.[14]

Our study had a larger sample size compared to previous studies. Since the method used for preparing paraffin-embedded tissue blocks can affect DNA and even destruct it, a beta-globin test (similar with the size of considered segment) was designed as a control to detect proper viral DNA purification. In this regard, only in one study, negative specimens for BK virus were considered as control for negative results.[15]

Incomplete deletion of paraffin from tissue blocks isasystematic error during PCR technique on beta-globin gene. We prepared deparaffinized solution and kept tissues in the heater for 24 hours. Since some reports have demonstrated that obtained JC virus DNA was higher in fresh frozen specimens compared to paraffin-embedded specimens, it is recommended to use fresh frozen specimens and needle-like specimens prepared from PCa specimens along with paraffin-embedded blocks.[24] Even so, Delbue and colleagues[22] reported that the efficiency of detecting BK virus DNA in FFPE tissue was significantly higher than frozen sections. They confirmed that quantitative real time PCR is the best gene assay method for detecting BK virus DNA in tissues and it supports the use of FFPE tissue specimens for molecular-based testing.

It seems that, problems such as tissue conservation

method, time duration before fixation of tissue, time duration in which the tissue is located in fixator, and finally lifetime of paraffin-embedded blocks can affect stability of viral DNA and also produce different results from various laboratories.[24] However, detecting viral genes due to the complete disappearance of the virus in tumor cells may depend on test sensitivity. This is because the detection rate of BK virus DNA in tumor tissues by quantitative real time PCR can be significantly higher than the rate obtained by both nested and regular PCR.[22] Therefore, using a conventional PCR kit and working with paraffin-embedded specimens were limitations of our study in detecting BK virus DNA.

## CONCLUSIONS

More BK virus infection was observed in PCa specimens compared to BPH specimens. Also, BK virus infection was predominantly observed in cancerous specimens with lower total Gleason scores. This may be in consistent to co factorial role of BKV in PCa triggering and hit and run theory. Simultaneous use of BK virus infection assessment methods, including serology tests for determining its antibody, measuring virus TAg in the cell, and also assessing the number of virus in the blood plus viral DNA in PCa cell, are also suggested future researches.

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