



Prevalence and genotyping of BK polyomavirus in kidney transplant recipients; a cross-sectional study in Chaharmahal and Bakhtiari province, Iran

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ABSTRACT

Introduction: BK polyomavirus (BKV) presents a significant challenge in renal transplantation, as it is strongly associated with nephropathy and subsequent graft loss. Although four genotypes have been identified based on VP1 region nucleotide sequences, genotype I remains the most prevalent globally.

Objectives: This study investigated the prevalence and viral subtypes of BKV among kidney transplant recipients in Chaharmahal and Bakhtiari province, Iran.

Patients and Methods: This is a cross-sectional, descriptive-analytical study. Urine samples were collected from 37 kidney transplant recipients. Viral DNA was isolated and analyzed using polymerase chain reaction (PCR). Phylogenetic analysis was performed using MEGA 6.0 software to identify viral subtypes and construct a phylogenetic tree.

Results: BKV DNA was detected in 17 of the 37 samples (46%). Sequence analysis identified subtype I as the dominant genotype in this region. While prevalence was higher in patients over 45 years of age, no statistically significant correlation was found between BKV positivity and age or gender.

Conclusion: Given the high prevalence of BKV in this cohort and the absence of specific antiviral therapies, routine pre- and post-transplant screening for both donors and recipients is strongly recommended to prevent BK virus-associated nephropathy (BKVN) and graft rejection.

Implication for health policy/practice/research/medical education:

This study emphasizes the importance of routine BK virus screening in kidney transplant recipients to prevent nephropathy and transplant rejection. It highlights the need for improved diagnostic protocols and resource allocation in transplant centers while providing valuable data for future research on BK polyomavirus management and subtype epidemiology.

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Introduction

Nephropathy remains a critical complication threatening kidney transplant survival. Major causes of graft dysfunction include acute and chronic rejection, recurrent primary disease, drug toxicity, ischemia-reperfusion injury, and opportunistic infections. Among infectious agents, BK polyomavirus (BKV) is the primary etiological factor for nephropathy and a leading cause of graft loss (1).

Primary BKV infection is typically asymptomatic and occurs early in life, with approximately 70% of the population becoming seropositive by 10 years of age.

Following primary infection, the virus establishes latency in the urothelium and renal tubular cells. Reactivation predominantly occurs in immunocompromised individuals, including HIV patients, pregnant women, and solid organ transplant recipients. While symptomatic infection may manifest with mild upper respiratory symptoms or fever, the virus usually remains in a latent, non-replicating state. Consequently, the detection of BKV in the blood is indicative of active viral replication (2-4). BKV reactivation occurs in 10% to 60% of kidney transplant recipients. Of these, 1% to 5% develop advanced nephropathy. Approximately 50% of grafts affected by BK

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virus-associated nephropathy (BKVN) are ultimately lost due to progressive renal damage (5).

The BKV virion consists of a non-enveloped, heat-stable capsid enclosing a double-stranded circular DNA genome of approximately 5.13 kb. The icosahedral capsid comprises three structural proteins; VP1, VP2, and VP3, with VP1 being the major capsid protein (6).

Based on sequence variations within the VP1 gene, BKV is classified into four genotypes. Genotype I is the most common globally, followed by genotype IV (7). Genotypes II and III are rare and have been reported in limited adult populations. Additionally, variations in the non-coding control region distinguish the virus into archetype (ww) and rearranged (rr) forms (8–11).

Previous studies detected that, transmission typically occurs by the respiratory tract, although transplacental, sexual, and blood, urine, or organ transplantation routes have also been confirmed. Diagnostic methods include urine cytology (Decoy cells), serology, histopathology (Haufen test), and molecular techniques such as PCR (12).

Objectives

Given the clinical significance of BKVN, monitoring BKV in both donors and recipients is essential for post-transplant management. This study aims to determine the prevalence and circulating subtypes of BKV among renal transplant recipients in Chaharmahal and Bakhtiari province, Iran.

Patients and Methods

Sample collection

This cross-sectional study included 37 kidney transplant recipients presenting to Ayatollah Kashani hospital in Shahrekord, Iran. All patients with a history of transplantation were eligible for inclusion. Due to the limited sample size, no exclusion criteria were applied. Approximately 50 ml of morning urine was collected in sterile containers containing 0.5 mL of 0.5 M EDTA (pH 8.0). Samples were transported to the Shahrekord university of medical sciences research center under cold-chain conditions. Following centrifugation, pellets were stored at -70 °C until analysis.

DNA extraction

Viral DNA was extracted using the Roche High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH, Germany) according to the manufacturer's instructions. DNA concentration and purity were assessed using a NanoDrop spectrophotometer (Germany).

Polymerase chain reaction

Conventional PCR (polymerase chain reaction) targeting the VP1 region was employed to detect the BKV genome (13). The primers used were: Forward 5'-GCC TGC AGC AAG TGC CAA AAC TAC TAAT-3' (nt 1630-1649) and Reverse 5'-GCA AGC TTG CAT GAA GGT TAA GCA

TGC-3' (nt 1956-1937). The 25 µL PCR reaction mixture contained 2.5 µL 10x PCR Buffer, 0.5 µL 10 mM dNTPs, 2.5 µL of each primer (50 pmol/µL), 0.15 µL Taq DNA Polymerase, 5 µL template DNA, and 14.85 µL sterile distilled water. The thermal cycling conditions were; initial denaturation at 95 °C for 2 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, extension at 72 °C for 30 seconds, and a final extension at 72 °C for 5 minutes. Then, PCR products were analyzed by electrophoresis on a 2% agarose gel at 100V for one hour and visualized under UV light. Positive controls were purchased from Keyvan Virology Specialty Laboratory, which consisted of previously laboratory-confirmed samples. Distilled water served as the negative control.

Sequencing and phylogenetics

Positive PCR products (327 bp fragment) were sequenced by Noorgene Genetics Laboratory (Ahvaz, Iran). Sequences were compared with reference strains using NCBI BLAST. Phylogenetic analysis and tree construction were performed using MEGA 6.0 software.

Statistical analysis

Data analysis was conducted using SPSS software. Descriptive statistics, including frequencies and percentages, were calculated. The chi-square test was conducted to evaluate the relationship between BKV prevalence and categorical variables (age, gender). A *P* value of less than 0.05 was considered statistically significant.

Results

Prevalence of BKV

Analysis of the 37 urine samples revealed the presence of the BKV VP1 gene (327 bp fragment) in 17 samples (46%), while 20 samples (54%) were negative (Figure 1).

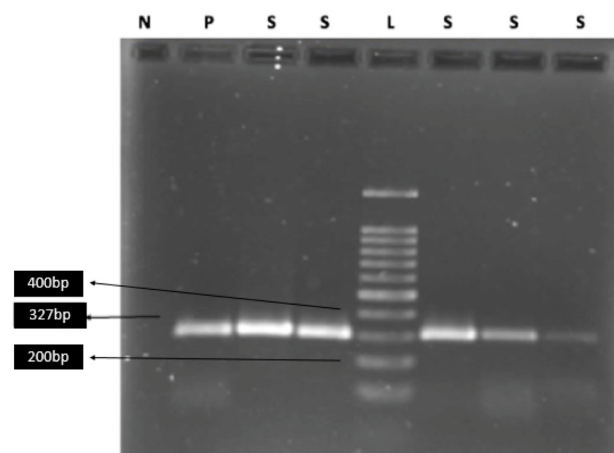


Figure 1. Agarose gel of PCR products: (N: negative sample, P: positive sample, L: Ladder and S: patient sample), The presence of a 327 bp fragment indicates successful amplification of the VP1 gene region.

Demographic associations

BKV prevalence was analyzed across age and gender groups. Although the infection rate was higher in patients over 45 years of age, this difference was not statistically significant ($P=0.54$). Similarly, gender was not significantly associated with viral prevalence, with rates of 45% in females and 46% in males (Tables 1 and 2).

Viral genotyping

Sequence analysis of the VP1 gene confirmed that all positive samples belonged to subtype I.

Discussion

BK polyomavirus is a common opportunistic pathogen in kidney transplant recipients, and its reactivation can lead to graft dysfunction. While BKV seroprevalence is high in the general population, the potential variation in pathogenicity among viral subtypes remains a subject of ongoing research. Currently, there are no approved specific antiviral therapies for BKV, although cidofovir has shown efficacy in inhibiting viral replication (14). Consequently, early detection via PCR is crucial to facilitate timely reduction of immunosuppression, which remains the primary management strategy.

Previous studies have suggested correlations between BKV reactivation and risk factors such as age, gender, and diabetes (15). In our study, we observed a BKV prevalence of 46%, with no significant difference between males (46%) and females (45%). These findings contrast with studies reporting gender disparities. This discrepancy may be attributed to our limited sample size or differences in specimen type (urine vs. plasma) (16). Consistent with

most literature, we observed a higher infection rate in patients over 45 years of age; however, this difference was not statistically significant. This aligns with findings by Zhong et al, who noted age-related trends that were largely dependent on sample heterogeneity (17).

Genotypically, our results are consistent with global epidemiological data. All isolates were identified as subtype I, the predominant subtype in Iran, Japan, Europe, and China (18,19). Phylogenetic analysis further confirmed that the isolates from Chaharmahal and Bakhtiari are closely related to strains from Kazakhstan and Japan, suggesting a likely origin from European and American lineages (13,17,18,20).

Conclusion

This study highlights a significant prevalence of BK polyomavirus (46%) among kidney transplant recipients in Chaharmahal and Bakhtiari province, identifying subtype I as the sole circulating genotype. Given the high risk of BKV-associated nephropathy and the lack of specific antiviral therapies, the implementation of routine pre- and post-transplant screening protocols for both donors and recipients is strongly recommended. Such measures are essential for the early detection of viral reactivation, allowing for timely intervention to prevent nephropathy and minimize the risk of graft rejection.

Limitations of the study

The primary limitation of this study was the small sample size ($n=37$), which necessitated broad inclusion criteria and precluded strict post-transplant time restrictions.

Table 1. Prevalence of BK virus among age groups

Age group (y)	Total	Positive	Negative	Relative abundance percentage
20-25	2	1	1	50
25-30	1	0	1	0
30-35	1	0	1	0
35-40	5	2	3	40
40-45	2	0	2	0
45-50	6	4	2	67
50-55	7	3	4	43
55-60	5	2	3	40
60-65	4	3	1	75
65-70	4	2	2	50
Total	37	17	20	46

Table 2. Prevalence of BK virus among genders

Gender	Total	Positive	Negative	Relative abundance percentage
Female	11	5	6	45
Male	26	12	14	46
Total	37	17	20	46

Authors' contribution

Conceptualization: Majid Shirani.
Data curation: Majid Shirani.
Formal analysis: Gholam Abbas Kaydani.
Funding acquisition: Majid Shirani.
Investigation: Parisa Dehghan.
Methodology: Gholam Abbas Kaydani.
Project administration: Gholam Abbas Kaydani.
Software: Gholam Abbas Kaydani.
Supervision: Majid Shirani.
Visualization: Parisa Dehghan.
Writing—original draft: Parisa Dehghan, Niloufar Afrough.
Writing—review & editing: Majid Shirani, Niloufar Afrough.

Conflicts of interest

The authors declare that they have no competing interests.

Declaration of generative artificial intelligence (AI) and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Gemini (Google) in order to improve the readability and grammatical accuracy of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Ethical issues

The research was conducted in accordance with the principles of the Declaration of Helsinki. The Ethics Committee of Shahrekord University of Medical Sciences approved this study. The institutional ethical committee at Shahrekord University of Medical Sciences approved all study protocols (Ethical code #IR.SKUMS.REC.1395.123). Accordingly, written informed consent taken from all participants before any intervention. This study was extracted from M.D thesis of Parisa Dehghan at this university (Thesis#1373). Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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