

DETECTION OF HUMAN POLYOMAVIRUS BK (BKV) IN BULGARIAN ADULT KIDNEY TRANSPLANT RECIPIENTS BY POLYMERASE CHAIN REACTION

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ABSTRACT

Reactivation of the polyomavirus BK (BKV) is increasingly recognized as a cause of renal-allograft dysfunction. Currently, patients at risk of nephropathy due to active BKV infection are identified by the presence of cells containing viral inclusion bodies ("decoy cells") in the urine or by biopsy of the allograft tissue. Demonstrating viral DNA by molecular methods in urine and/or blood is emerging as a non-invasive tool for virology diagnosis and patient management. We report here the development of an in-house polymerase chain reaction (PCR) assay for BKV and its application for detection of BKV DNA in 43/50 (86%) of the urine samples, and in 4/50 (8%) of the peripheral blood leukocytes (PBLs) samples collected from adult kidney transplant recipients between 30 and 70 years of age. Testing for BKV in urine and PBLs from renal-allograft recipients by the use of the PCR is sensitive and specific method for identifying the patients at risk of viral nephropathy.

Keywords: Human polyomaviruses, BK virus, BKV, Renal transplantation

Introduction

The human polyomavirus BK (family *Polyomaviridae*) is ubiquitous viral agent that infects a large proportion of healthy individuals. Primary infection occurs during childhood and is usually unapparent, but the virus establishes latent infection in renal tissues and B-lymphocytes, and a BK virus-related disease can be developed under conditions of severe cellular immunosuppression, such as organ transplantation or hematological malignancies. Reactivation of BK virus (BKV) infection is related to urinary tract diseases, such as hemorrhagic cystitis, ureteric stenosis, glomerulonephritis, and graft nephropathy, which are most commonly found in transplant patients undergoing immunosuppressive therapy (9).

In renal transplantation, BKV was initially connected with the post-transplant ureteric stricture, but now is recognized as a possible cause of transplant interstitial nephritis, mimicking rejection or drug toxicity. BKV may contribute to allograft dysfunction in up to 8% of renal transplant patients (13) resulting in graft loss in 45% of these cases (7). There are currently no described epidemiologic factors for this disease process, and it is unknown whether this is an acquired infection with the graft or whether it represents recipient reactivation with immunosuppression. The first cases of BKV-nephropathy in renal transplant patients were reported in the 1970s (4). However, a significant incidence of BKV infection in the renal transplant population has only been seen in recent years. A reemergence of this pathogen became evident after 1995 (10) and was confirmed by several subsequent reports from different centers (11). The exact reason is not clear, but there is general concern that the widespread use in BIOTECHNOL. & BIOTECHNOL. EQ. 21/2007/3

most transplantation centers of the new immunosuppressive agents (tacrolimus, mycophenolate mophetic) increases the risk of viral reactivation (8).

The methods used for the detection of BKV viruria include isolation in cell culture, electron microscopy, cytological examination and immunofluorescent staining of exfoliated urinary cells, ELISA for antigen, and DNA hybridization with labeled nucleic acid probes (9). Several studies have shown PCR to be an effective tool for detecting polyomaviruses in a range of clinical samples (1, 14). As a result, PCR is now emerging as the method of choice ("gold standard") for the identification of BKV in clinical specimens and the patients, who are at risk for development of BKV-related diseases.

In this communication we report the development of an in-house PCR assay for BKV and its application for detection of the virus in urine and peripheral blood cells. This test was successfully used for the detection of BKV genomes in different samples of adult renal graft recipients.

Materials and Methods

Clinical specimens and DNA preparation

Urine and blood samples were collected from 50 adult kidney transplant patients (between 30 and 70 years of age) at the Clinic of Nephrology and Transplantations, Medical University of Sofia. First or second voided urine was preferred and 50 ml containers supplied with 5 ml of absolute ethanol, which prevents from bacterial growth and destruction of the urothelial cells were used (12). Blood samples were collected using vacutainers provided with anti-coagulant EDTA (Becton-Dickenson, USA). Peripheral blood cell fraction was separated from the whole blood by the use of the Lymphoflot reagent (Biotest, Germany; Cat.No. 824013). DNA extraction from both materials was carried out with the spin column-based QIAmp

DNA minikit (QIAGEN, Courtaboeuf, France), according to the manufacturer's instructions. The final extraction volumes of 50 µl were then stored at -70°C until their use in the PCR procedures.

PCR for detection of BKV. An in-house PCR protocol for the detection of the BKV was carried out with the primer pair previously designed in our laboratory: 5'-ATC CAG CCT TTC CTT CCA TT-3' (nt 2992-3011) and 5'-CTG TCC CTA AAA ACC TGC CAA-3' (nt 3090-3211). Target sequences were amplified in total volume of 50 µl containing 0.5 µg of DNA and reaction mix (50 mM KCl, 1.5 mM MgCl₂, 200 µM of each dNTPs, 30 pmol of each primer, and 1.25 U of thermostable *Taq* polymerase (Invitrogen, USA). After initial heating at 94°C/5 min, 40 cycles of amplification were carried out as follows: denaturation at 94°C/30 sec, annealing at 60°C/30 sec, and extension of the primers at 72°C/1 min. After the last cycle the samples were incubated at 72°C for additional 10 min to complete the extension of the primers. Amplifications were performed on a PTC-150 minicycler (MJ Research, USA). To fractionate the amplicons electrophoretically, 20 µl of the PCR product were mixed with 4 µl 5X loading buffer and run alongside with a 100 bp DNA Ladder MW marker (Invitrogen, USA) on a 2% agarose gel containing 0.5 mg/ml ethidium bromide. Running conditions were: 100 mV for 80 min at room temperature.

Results and Discussion

BKV DNA was detected by our in-house PCR assay in 43 (86%) of the 50 tested urine specimens. All positive samples revealed abundant amplicons of 215-bp indicative of large T-gene of the BK virus genomes (**Fig. 1**). Negative results were obtained for 7 samples.

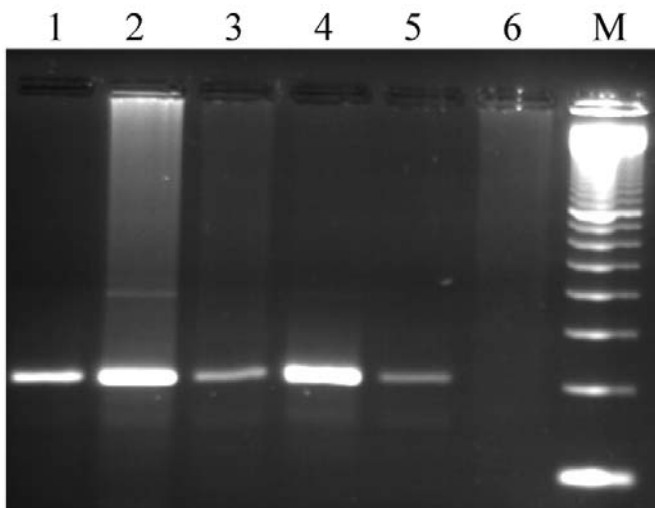


Fig. 1. PCR-amplified BKV DNA fragments (large T-gene) in urine samples from renal-allograft recipients on 2% agarose gel. Lines 1-5: positive samples; Line 6: negative sample; Lane M contains size markers (100-base-pair ladder)

BK virus DNA was found in only 4/50 (8%) of the PBLs samples of the renal-allograft recipients.

All patients with active BKV replication presented variable clinical manifestations (chronic and acute renal insufficiency, glomerulonephritis or pyelonephritis). Generally, there was frequent shedding of BKV in the urine of the allograft patients, which could be possibly related to various reasons. It is stated, that the augmented immunosuppression, can lead to continuous viral replication and shedding of the virus in urine (8). All of the tested patients were subjected to immunosuppressive anti-rejection regimens, consisting of different medication schemes. This could provide in combination with the suppressed cell-mediated immunity, appropriate environment for viral replication thus leading to BKV-related diseases (5). It is important to state that immediately after renal transplantation, there is, also clinically silent BKV viremia, preceding the development of BKV nephropathy, another possible reason for the increased viral shedding (2). Nevertheless, BKV viremia is indicative of activated infection and partly could be connected with some of the clinical manifestations – especially chronic and acute renal nephropathy, where is expressed opinion that viral reactivation may play pivotal role (5). Of more importance, data show that reactivation and replication of BKV lead not only to viremia, but also to tubulointerstitial nephritis, which can lead to severe allograft dysfunction and graft loss (8). Reactivation of BKV infection was demonstrated from 10 to 68% in renal transplant recipients (5). Only a limited proportion of them (1-10%) progress from reactivated infection to histologically manifest polyomavirus BKV nephropathy (6). That is why, detection and quantification of BK virus DNA in plasma by PCR is potentially useful for the identification of patients with clinically significant BKV reactivation, but these methods have yet to be standardized. Unfortunately, no routine screening is available for this virus, so this complication is probably underestimated.

Our results showed limited detection rates in peripheral blood cells – only 4/50 positivities or 8%. Lymphoid cells are proposed to be a site of polyomavirus latency, as their sequences have been frequently found in blood cells, but recent data show that PCR on PBLs is as well indicative for BKV recent infection or reactivation (3). The presence of circulating virus is associated with active nephropathy because the virions enter the circulation through peritubular capillaries following tubular damage (2). Thus demonstrating BKV DNA in blood could be surrogate diagnostic marker to urine PCR for diagnosis confirmation and further patient's monitoring. The small percentage of blood positivities, as compared to urine PCR results, shows that limited number of patients could be at potential risk at developing BKV nephritis. It seems that in most of the cases viremia is not linked to distinct pathologic entity, and testing for BKV DNA in blood combined with urine PCR has much higher clinical significance (8).

In addition to high sensitivity and specificity, the short turnaround time from the receipt of the specimen to the final result makes PCR attractive for diagnostic use. Results can be obtained on the day of or the day after specimen arrival. Processing of the urinary cell pellet by boiling, instead of DNA

extraction, shortens the time required to perform PCR and also eliminates the risk of cross-contamination of specimens during the extraction procedure. This technique is also particularly well-suited for the testing of many samples simultaneously, a distinct advantage for epidemiological studies.

In conclusion, human polyomavirus BK is related to different clinical manifestations among renal-transplant patients. The routine use of PCR on urine and PBLs by PCR is a useful tool for the rapid and sensitive detection of reactivated BKV infection. Thus, establishing instant diagnosis may be of great value for monitoring the patients, who are at possible risk for the development of BKV nephropathy.

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