



## Monitoring subtypes of the human polyomavirus BK in Iranian adult kidney transplant patients

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### ABSTRACT

BK virus (BKV) is a polyomavirus with seroprevalence in adults, ranging from 60 to 100%. It is considered as usual cause of renal dysfunction after the allograft renal transplantation nephropathy. Potent immunosuppressive therapy in kidney transplantation can lower the rate of acute rejection. Therefore, untreated BKV infections lead to kidney allograft dysfunction or loss. In order to estimate the difference, this study investigated the BKV in urine samples of kidney transplant patients. In this study, we used 220 urine samples from allograft recipients in the time period of 2010-2013. Then, the 287 bp typing region and the PCR increased from the urinary DNA. The PCR products were digested by three limitation enzymes, namely AluI, Cfr13I and RsaI to determine the BKV subtypes. The BKV subtypes are common in the city of Esfahan, Iran. This research showed that 102 (75%) samples were infected by BKV type I. 7 (5%) and BKV subtypes II, 5 (4%) III, and 22 (16%) IV were found in our patients. On the other hand, mixed infections did not clear in the recipients. Our findings showed that BKV replication might occur after kidney transplantation and through the early hours. BKV types II, III and IV are brand new in Iran and previously were not apparent in samples of urine in different kidney transplant patients.

### 1. Introduction

In 1971, the BK virus was first isolated from a kidney transplant recipient with an ureteral structure. The BK virus (BKV) is a polyomavirus with a circular DNA genome of approximately 5,300 bp. BKV causes ubiquitous infection in early childhood, with seroprevalence in adults, ranging from 60 to 100%. Primary infection presumably occurs during childhood via a fecal-oral or a respiratory route, and is usually asymptomatic (Gardner et al., 1971; Tremolada et al., 2010). After primary infection, the BKV persists in a latent state

in cells of several organs including kidney (the main site of BKV latency in healthy individuals), peripheral blood leukocytes, and other sites such as the lung, eyes, liver, and brain (Anzivino et al., 2011).

The BK infections are an increasing, and it is a common cause of renal dysfunction following the allograft renal transplantation (Maximilian et al., 2012). The BK virus-associated nephropathy (BKVAN) is one of the main causes of allograft loss in kidney transplant recipients. Quantitative polymerase chain reaction (PCR) of the BK virus

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DNA (BK DNA) can be more sensitive and specific.

The BKV appears to cause clinical disease only in individuals with changed or altered immune responses including in the pregnant women with human immune deficiency virus-1 (HIV-1) infection or individuals who did chemotherapy, and in bone marrow and solid organ transplant recipients (Barraclough *et al.*, 2011). The BKV nephropathy currently affects 1-7% of recipients and has been associated with 10–100% graft loss rate depending on the severity of histological involvement (Memon *et al.*, 2012). Transmission of the BKV occurs via body fluids (Motazakker *et al.*, 2012).

Nephropathy from the BKV infection is a challenge in kidney transplant recipients. It can be the result of modern potent immunosuppressant purposed at declining acute rejection and improving allograft survival. Untreated BKV infections cause kidney allograft dysfunction or loss. Decreased immunosuppressant is the main therapy, but predisposes to acute and chronic rejection. Screening protocols for early detection and prevention of symptomatic BKV nephropathy yield best results. Polyomavirus infection in kidney transplant recipients is an interesting research topic. However, in 1971 the two human polyoma viruses; the BKV and the JC virus (JCV), were reported to have minor effects (Geraldine *et al.*, 2008; Padgett *et al.*, 1971). In a study, the kidney transplant recipients were treated with prednisone and azathioprine in early 1980s. Also, they formed the basis to figure out the polyomaviruses in transplant recipients. The Rituximab and intravenous Ig (IVIG) are generally used for desensitization of the HLA and blood group-incompatible (ABOi) transplants. On the other hand, serious infection related with the rituximab administration was reported (Kahwaji *et al.*, 2011). BK is the main etiologic agent of the polyomavirus-associated nephritis occurring in up to 10% of renal transplant recipients (RTRs) (Hirsch *et al.*, 2002; Bohl *et al.*, 2007).

Polyomavirus-associated nephropathy (PVAN) is a major problem after the renal transplantation that is stimulated by reactivation of the human polyomavirus BK Polyomavirus (BKV) (Tremolada *et al.*, 2010). The polyoma virus infection results in nephropathy after the kidney

transplantation, but has not been entirely considered as the innonrenal organ transplantation (Thomas *et al.*, 2009). Currently, the rates of acute rejection in kidney transplantation have reduced, but it has caused the emergence of BKV-associated nephropathy (BKVAN). In this study, we investigated BKV in the urine samples in a cohort of kidney transplants to find out their probable difference (Anzivino *et al.*, 2011).

## 2. Materials and Methods

### 2.1. Clinical Specimens Collection and Processing

This study was carried out on a total of 220 urine samples, collected from a cohort of adult renal allograft recipients at different times after transplantation. The hepatitis C, B, and CMV tests were negative according to the serological items.

### 2.2. Urine Samples

Total of 220 Urine samples were collected from the kidney transplant patients from the research Park laboratory (Esfahan, Iran). No patient had active graft rejection episodes or developed nephropathy during the study. About 20 ml of urine was collected in a 50 ml plastic tube.

### 2.3. DNA Extraction

One milliliter of urine was centrifuged for 2 minutes. The sediment of samples were washed in 1 ml PBS, and centrifuged for 2 min. Then, the DNA was extracted using the high Pure Viral Nucleic Acid Kit (Roche, Germany). After the addition of 200 µl of Binding Buffer and pouring it in High Pure filter tube, the DNA was centrifuged and eluted 50 µl with the elution buffer. Finally, absorption of the DNA solution was measured at 260 nm.

### 2.4. PCR

The 287 bp region spanned from 1650 to 1936 nt in the BKV (Dunlop) genome (GenBank accession no. V01108; NCBI no. NC\_001538), and contained the whole effective sequence within the 327 bp typing region (Jin *et al.*, 1993b). The primers were used to amplify the typing region were the 327-1PST (5'GCC TGC AGC AAG TGC CAA

AAC TAC TAA T-3'nt 1630-1649) and the 327-2HIN (5'GCA AGC TTG CAT GAA GGT TAA GCA TGC-3'; nt 1956-1937) (Zhong *et al.*, 2007; Jinj *et al.*, 1993). The total reaction volume of 25  $\mu$ l contained 5  $\mu$ l of crude viral DNA, 1.5 U DNA polymerase (FERMENTAZ), 200  $\mu$ M of dNTP, 50 pmol of each primer and PCR buffer, supplied by the manufacturer. After activation at 95°C for 2 min, the amplification reaction was performed for 40 cycles. The cycle profile was 94°C for 1 min, 55°C for 1 min and 72°C for 2 min. The amplification was carried out in a CORBET thermal. The expected amplicon was 327 bp long that covers the type-specific region. The PCR product was evaluated via electrophoresis on a 3% agarose gel. Sequences of 287 bp typing fragments were available for several BKV isolates belonging to I-IV (Takasaka *et al.*, 2004). The PCR products digested by three restriction enzymes; AluI, Cfr13I and RsaI, can be used to identify the BKV subtypes. The RFLP analysis involving these enzymes was carried out as follows; 2.5  $\mu$ l aliquot of a purified PCR product was digested at 37°C for 1 h with 10-20 U of each enzyme. The digest was resolved by electrophoresis on a 3% agarose gel stained with ethidium bromide. A 327 bp fragment containing the 287 bp typing region was PCR-amplified from each BKV subtype as described earlier. Table 1 represents the sizes of sub fragments detected after digestion of the amplified fragment with indicated restriction enzymes.

**Table 1.** Fragment patterns for indicated subtypes (bp).

Restriction enzyme	I	II	III	IV
AluI	193	193	342	342
	149	149	-	-
Cfr13I	245	245	245	342
	97	97	97	-
RsaI	294	342	212	342
	48	-	130	-

### 3. Result

BKV DNA was detected in 220 urine samples in total. We determined the subtypes of these BKV DNAs RFLP analysis. Using the PCR to amplify the 287 bp typing region, we screened 220 urine samples using the BKV DNA.

There was no significant association between the detection of BKV in urine among the recipients' characteristics (age, gender, time of dialysis, etiology of renal failure and immunosuppressive therapy); donor parameters (age, gender, weight, BMI and cause of death), or graft feature (score and ischemic time) ( $P > 0.05$ ). The BKV subtypes are prevalent in Esfahan, Iran. This research showed that 102 (75%) samples were infected by BKV type I, 7 (5%) and BKV subtypes II, 5(4%) III, and 22(16%) IV was found in our subjects. Mixed infections were not detected in the recipients.

A number of cases of affliction with these bacteria and the relationship of the infections with premature delivery were studied. Among the 44 sample cases, 3 cases of abortion and one case of premature delivery were reported. In the one case of premature delivery, no bacteria were identified. However, the presence of *Mycoplasma* was reported in the three cases of abortion. The only significant relationship was between the existence of infection with *Mycoplasma genitalium* and the abortions ( $P < 0.05$ ). Infections caused by *Gardnerella* and *Neisseria* did not show any significant relationship despite the premature delivery and abortion.

### 4. Discussion

Standard immunosuppressive regimens in renal transplantation generally include calcineurin inhibitors (CNIs), tacrolimus or cyclosporine, mycophenolate (mycophenolate mofetil [MMF] or enteric coated mycophenolate sodium), and corticosteroids (methylprednisolone or prednisolone). In the past 50 years, advanced, more potent immunosuppressive agents have been found related to higher prevalence of polyomavirus-associated nephropathy (PVAN) and BK polyoma virus-associated nephropathy (BKVAN) in renal transplant patients, showing the relationship between the human polyomavirus BK (BKV) reactivation and the failure of immune system. Solid organ transplantation is recognizable as a key lifesaving procedure of terminal organ failure, therapy of select based on patients' quality of life. BKV is the only primate polyomavirus that has subtypes distinguishable by immunological reactivity (Knowels *et al.*, 2006). The distribution of BKV subtypes within the human population was

previously studied in England, Tanzania, the United States, Japan and Germany (Transplantation. Author manuscript; available in PMC 2010 August 15) (Jinj *et al.*, 1993). A previous serological study revealed four BKV serotypes (Knoweles *et al.*, 1989). Subtype I is the most prevalent, followed by the subtype IV, with the subtypes II and III occurring less frequently. The BKV subtype IV was previously found prevalent in East Asia, except for Japan (Boukhoum *et al.*, 2011). Nevertheless, there is evidence that the prevalence of this subtype was underestimated in other regions (Krumbholz *et al.*, 2008).

Subtypes I, II, III and IV were detected in 70, 0, 2-3 and 10 % of the BKV-positive patients, respectively (Nishimoto *et al.*, 2007). A research in Iran on 334 patients in the range of 11+3 reported 27% of Virusin (Sharifian *et al.*, 2009). Also, in 2012 216 percent of BKV was recognized in 31 patients. At that time, only subtype I was reported among the Iranian Turkish kidney transplants (Motazakker *et al.*, 2012).

In this study, we screened 220 urine samples obtained from the kidney transplant patients at the research Park laboratory (Esfahan, Iran). The PCR was used to find the presence of BKV DNA. The findings showed that 102 (75%) samples were infected by the BKV type I. 7 (5%) BKV subtypes II, 5 (4%) III, and 22 (16%) IV was found in our patients. BKV types II, III and IV are novel in Iran and this study could detect them for the first time in urine samples of kidney transplant patients. In order to support the results, the study should be conducted on larger patient populations with longer follow-up period.

## Conclusion

Our results confirm the findings of previous studies, showing that the BKV replication may happen during early hours after the kidney transplantation. The highest occurrence is in the third post-transplantation month and then it declines within sixth months due to the induction therapy. Moreover, monitoring the BKV viremia and viruria enables identification of renal transplant patients at the risk of BKVAN. Viral re-activation may take place at any time point, although it is more likely to start in the early post-transplantation period, e.g. the

first post-transplantation hours as a result of the induction therapy.

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