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Comparison of Detecting Methods of BK Virus Infection in Patients with Renal Allograft Recipients

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Background : BK virus nephropathy (BKVN) is an emerging problem as a consequence of the use of potent immunosuppressive agents. Because optimal detection methods for the diagnosis of BKVN are required clinically, we compared the results of renal allograft biopsy, urine cytology, and urine and blood viral loads. **Methods :** Four hundred sixty two case notes from 2004 to 2009 at Seoul St. Mary's Hospital were reviewed. During that period, 286 cases of urine cytology for decoy cells, 938 cases of urine BKV reverse transcription-polymerase chain reaction (RT-PCR), and 1,029 cases of blood BKV RT-PCR were performed. All diagnostic methods were performed in 85 cases. **Results :** A histological diagnosis of BKVN was made in 2.4% of cases (11/462). Urine cytology for decoy cells was positive in 26.2% (75/286). BKV RT-PCR revealed viruria in positivity of 22.1% (207/938) and viremia in 5.2% (54/1,029). In cases of BKVN, the sensitivities of urine and blood BKV RT-PCR were all 100% and the specificities were 69% and 94.5%, respectively. In cases with positive urine decoy cells, the sensitivities of urine and blood BKV RT-PCR were 50% and 27.7%, with specificities of 77.7% and 100%, respectively. **Conclusions :** BKV screening by RT-PCR assays may be a clinically useful noninvasive test to identify renal recipients with concurrent BKVN.

Key Words : Kidney transplantation; BKV-associated nephropathy; Real-time PCR; Decoy cells; Urine cytology

During the past decade, polyomavirus associated nephropathy (PVAN) has emerged as a significant cause of allograft failure after renal transplantation.[1] Human polyomaviruses are DNA viruses that manifest as a respiratory illness or as an asymptomatic infection in immunocompetent individuals. After primary infection, polyomaviruses maintain latency in the kidneys and urogenital tract.[1] In immunocompromised hosts, reactivation of polyomaviruses may occur and cause hemorrhagic cystitis, ureteral stenosis, and PVAN.[1] The vast majority of PVAN is caused by the BK virus (BKV), and the JC virus (JCV) is responsible for less than 3% of cases.[1,2] Currently, reduction in pharmacological immunosuppression is the mainstay of therapy; however, the success of this approach relies upon the early diagnosis of the BKV infection.[3] Early detection of a BKV infection within this stage provides a preemptive treatment strategy, because asymptomatic viruria and viremia precede BKV nephropathy (BKVN). Although, urine cytology for decoy cells and a demonstration of viruria or viremia with the polymerase chain reaction (PCR) for BKV DNA are common screening tools, there are some discordances in these methods. Urine cytology, despite being less expensive than PCR assays, has a lower positive predictive value (PPV) due to high interobserver variability.[4] Renal biopsy, regarded by most as the definitive diagnostic tool, can sometimes produce a negative result because of a tissue sampling error. Viral load monitoring by quantitative BKV DNA tests is a highly sensitive tool to monitor, diagnose, and manage a BKV infection;[5] however, a false positive reaction is also reported in as many as 1.8 to 3.6% of cases.[6]

In this study, we present our experience with BKV infection in 357 renal transplant patients with allograft biopsy proven results compared with urine cytology and quantitative reverse transcription (RT)-PCR on urine and blood.

MATERIALS AND METHODS

Patients and samples

A total of 462 allograft biopsies from 357 renal transplant recipients were enrolled between March 2004 and February 2009 at Seoul St. Mary's Hospital. Clinical screening for polyomavirus infection consisted of a urine cytological evaluation for decoy cells and quantitative urine and blood RT-PCR for BKV DNA. Urine cytology was reviewed for decoy cells in 286 cases from 216 renal transplant recipients clinically suspicious of BKVN. For the BKV RT PCR, 938 urine samples from 395 recipients and 1,029 serum samples from 418 recipients were studied (Table 1). All diagnostic methods for BKV, renal biopsy, urine cytology, urine and blood BKV RT-PCR, were performed in 85 cases. Of them, renal biopsies with urine and blood BKV RT-PCR were performed at the same time in 57 cases. Urine cytology with urine and blood BKV RT-PCR were performed simultaneously in 81 cases. The remaining cases were excluded because of unmatched time intervals between each procedure.

Table 1. Diagnostic methods for detecting a BKV infection and BKVN in renal transplant patients

Test	No. of patients tested	No. of patients with positive result
Urine cytology	286	75
Urine BKV RT-PCR	938	207
Serum BKV RT-PCR	1,029	54
Allograft renal biopsy	462	11
SV40T Ag staining	462	11

BKV, BK virus; BKVN, BK virus nephropathy; RT-PCR, reverse transcription-polymerase chain reaction.

Allograft biopsies were conducted by protocol at 14-day routine follow-up visits and, where indicated, to investigate declining allograft function. BKVN was diagnosed from formalin-fixed paraffin-embedded 4-μm sections, by the presence of intranuclear viral inclusion bodies in epithelial cells and the immunohistochemical detection of SV40 antigen in renal biopsy specimens (Fig. 1).

Urine cytology samples were stained using the Papanicolaou method and examined for the presence of urine decoy cells (characterized by a ground-glass appearance with an enlarged nucleus, occupied by a basophilic inclusion surrounded by chromatin) (Fig. 2).

BKV-DNA quantification was performed by RT TaqMan PCR (Applied Biosystems, Foster City, CA, USA) to detect the target viral capsid protein-1 (*VP-1*) gene encoding BKV using the ABI PRISM® 7000 Real-Time PCR System (Applied Biosystems). According to Viscount *et al*.,[7] levels of BKV DNA greater than 5.0×10^3 copies/mL are reproducible. Therefore, a viral load of $< 1.0 \times 10^4$ copies/mL was considered negative for this study because of a lack of reproducibility (i.e., not always detectable on repeated testing).

RESULTS

Prevalence of decoy cell shedding, BKV viruria, and BKV viremia

Urinary decoy cell shedding was detected in 75 (26.2%) of 286 cases. BKV viruria was observed in 207 (22.1%) of 938 cases

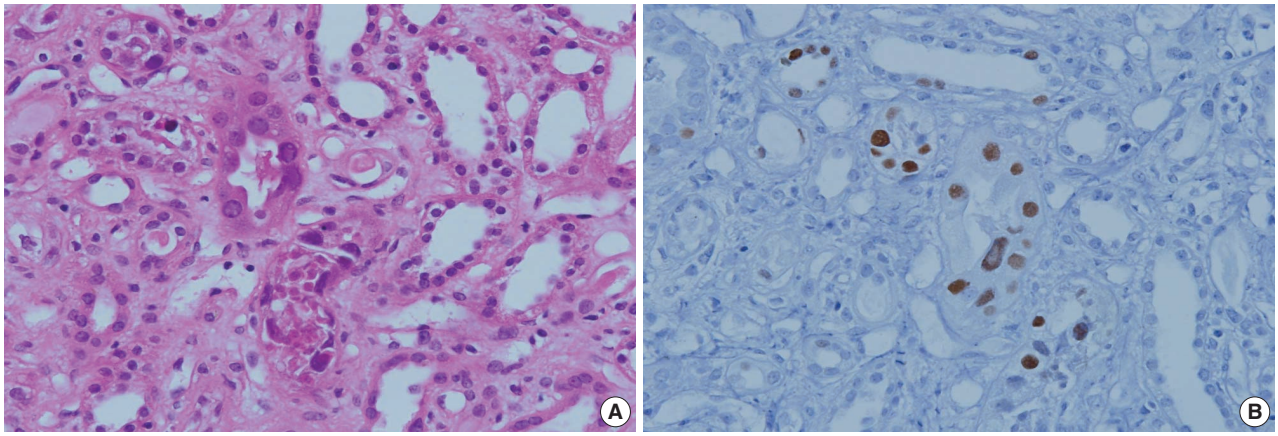

Fig. 1. (A) Several infected cells show large homogeneous nuclear inclusions surrounded by a thick nuclear membrane. (B) The SV40T immunostain is strongly nuclear positive in the infected cells.

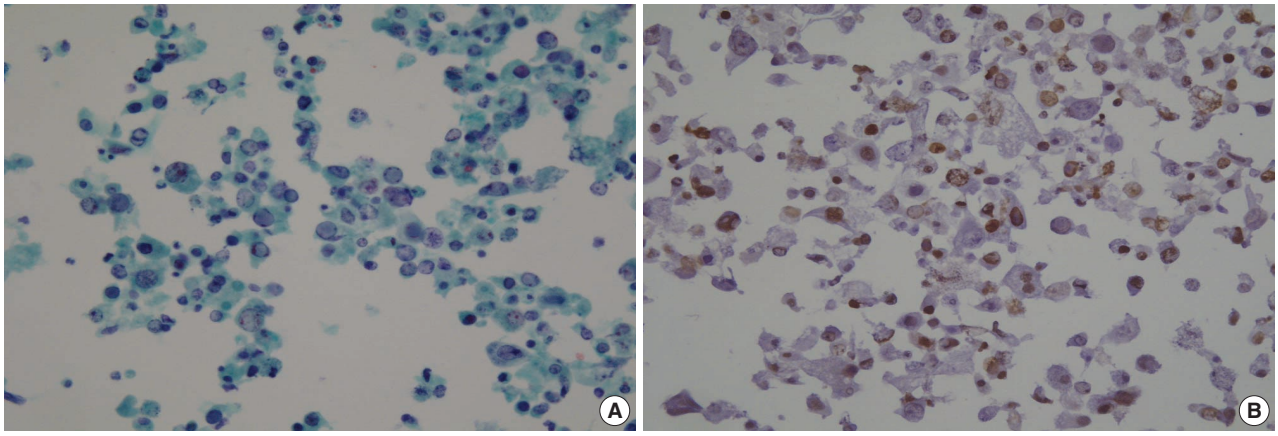

Fig. 2. (A) Viral cytolysis leads to shedding of tubular cells into the tubular lumen containing large amphophilic inclusions with a ground-glass appearance. (B) The SV40T immunostain is strongly nuclear positive in the infected tubular cells, which have enlarged nuclei corresponding to intranuclear inclusions.

Table 2. Characteristics of urine and blood BKV RT-PCR as predictors for BKVN in renal biopsy samples (n = 57)

BKV RT-PCR	Sensitivity (%)	Specificity (%)
Urine	100 (2/2)	69 (38/55)
Blood	100 (2/2)	94.5 (52/55)

BKV, BK virus; RT-PCR, reverse transcription-polymerase chain reaction; BKVN, BK virus nephropathy.

Table 3. Characteristics of urine and blood BKV RT-PCR as predictors for BKVN in renal biopsy samples (n = 57)

BKV RT-PCR	PPV (%)	NPV (%)
Urine	10.5 (2/19)	0 (0/38)
Blood	40 (2/5)	100 (52/52)

BKV, BK virus; RT-PCR, reverse transcription-polymerase chain reaction; BKVN, BK virus nephropathy.

Table 4. Characteristics of urine and blood BKV RT-PCR as positive predictors for decoy cells in urine cytology samples (n = 81)

BKV RT-PCR	Sensitivity (%)	Specificity (%)
Urine	50 (9/18)	77.7 (49/63)
Blood	27.7 (5/18)	100 (63/63)

BKV, BK virus; RT-PCR, reverse transcription-polymerase chain reaction.

Table 5. Characteristics of urine and blood BKV RT-PCR as positive predictors for decoy cells in urine cytology samples (n = 81)

BKV RT-PCR	PPV (%)	NPV (%)
Urine	39.1 (9/23)	84.5 (49/58)
Blood	100 (5/5)	82.9 (63/76)

BKV, BK virus; RT-PCR, reverse transcription-polymerase chain reaction.

and BKV viremia in 54 (5.2%) of 1,029 cases (Table 1).

Prevalence of biopsy-proven BKVN

A total of 462 renal allograft biopsies were taken from 357 patients. BKVN was diagnosed in 11 (2.4%) of 462 cases (Table 1).

Concurrence of biopsy-proven BKVN with decoy cells, BKV viruria and BKV viremia

Using biopsy-proven BKVN as the gold standard for the diagnosis of a BKV infection and the lowest limit of detection (1.0×10^4 copies/mL) as the RT-PCR assay threshold, sensitivities of the urine and blood BKV RT-PCR for BKVN were 100% and the specificities were 69% and 94.5%, respectively (Table 2). The PPV of the urine and blood BKV RT-PCR for BKVN were 10.5% and 40%, and the negative predictive values (NPV) were 0% and 100%, respectively (Table 3).

Concurrence of decoy cell shedding with BKV viruria and BKV viremia

Urine decoy cells were identified in 21/81 cases (25.9%). Using the presence of decoy cells as the standard for the diagnosis of a BKV infection, the BKV viruria threshold of $> 1.0 \times 10^4$ copies/mL had a sensitivity of 50% and a specificity of 77.7% for predicting concurrent decoy cell shedding. In contrast, a BKV viremia threshold of $> 1.0 \times 10^4$ DNA copies/mL predicted, the presence of decoy cell shedding with a 27.7% sensitivity and a 100% specificity (Table 4). The PPVs of the urine and blood

BKV RT-PCR for urine decoy cells were 39.1% and 100% and the NPVs were 84.5% and 82.9%, respectively (Table 5).

DISCUSSION

Since the first case was diagnosed by Purighalla *et al*.[8] in 1995, BKVN has emerged as a significant cause of graft dysfunction and even failure in renal allograft patients. The gradually increased prevalence of BKVN from 1% in 1995 to 5% in 2001 and 8% in 2007 is likely due to the use of potent immunosuppressive drugs, particularly tacrolimus, mycophenolate mofetil, and a steroid combination.[9] BKVN occurs more frequently during the first year posttransplantation.[9] In one study, the incidences of viruria and viremia at 1 year were 35% and 11.5%, respectively, compared with 17% and 3% at a mean time of 49 months posttransplantation.[10] Although managing a BKV infection includes reducing immunosuppression alone or combined with antiviral therapy, such as cidofovir or leflunomide, only an early diagnosis and reduction of immunosuppression reliably improve graft survival.[3,10]

At present, several methods are used to diagnose a BKV infection. Urine cytology for decoy cells and the demonstration of viruria or viremia with PCR for BKV DNA are common screening tools.[5] Urine cytology is inexpensive and displays a high negative predictive value, but polyomavirus shedding in urine is very common and occurs in 20% to 45% of renal transplant recipients.[7] In our data, urinary decoy cell shedding was positive in 26.2% of allograft recipients. Thus, the predictive characteristics of urinary decoy cell shedding are limited, and distinguishing between the BK and JCV cannot be made based on urine cytology but requires performance of urine PCR.[4,11] A combination of significant viremia and viruria leads to a diagnosis of presumptive BKVN.[4]

Given these considerations, a quantitative assay with high predictive value for BKVN would be of clinical value. In this context, quantitative PCR assays have been proposed to screen renal transplant recipients for BKV infection. According to some studies, BKVN is predictable by PCR; the PPV of urine BKV PCR is about 98% and the PPV of blood viral DNA quantification is 70%.[12,13] Our study indicates a superiority of urine and blood RT-PCR compared to urine cytology for identifying concurrent BKVN. While the presence of decoy cells in urine was neither highly sensitive nor specific for BKVN, detecting BKV DNA in the urine or plasma was 100% sensitive. Our results also demonstrated that blood BKV RT-PCR provided a superior PPV and NPV compared to urine BKV RT-PCR when performed as a screening test for BKVN.

Electron microscopy studies may be useful for diagnosing and confirming a BKV infection based on the different ultrastructural morphology of the most common viral infections, including adenovirus, herpesvirus, and CMV inclusions.[14] However, this method is less practical than immunohistochemical studies because of its lower sensitivity and higher cost.[15]

A definitive BKVN diagnosis can only be made histologically by demonstrating typical viral cytopathic findings specifically seen in the renal tubular epithelium and urothelium.[16] Cellular degeneration related to the viral infection itself can make the identification of viral changes more difficult. Currently, to make a definitive BKVN diagnosis, the most widely used confirmatory method is immunohistochemical staining for SV40T-Ag, which identifies all polyoma virus infections due to cross-reactivity between SV40 and both BKV and JCV.[16,17] In this study, we performed SV40T-Ag immunostaining for all renal biopsy samples to overcome the limitation of histopathology, and the positive reaction was well correlated with viral cytopathic changes, including intranuclear inclusions. Furthermore, this reaction is very helpful when making a definitive diagnosis, especially in cases combined with acute rejection. The sensitivity of histopathology could be limited because of focal involvement of BKVN, especially during the early stage of infection and the possibility of tissue-sampling errors. However, the requirement of a tissue evaluation in patients with suspected BKVN remains, considering that a renal biopsy is necessary to exclude other pathological processes, such as acute rejection that may coexist.[17,18]

Considering this and the focal nature of renal involvement, BKV DNA quantitation could be useful with little evidence of viral cytopathy.[19] In one study, quantitative BKV PCR demonstrated asymptomatic viruria in 30% of samples from renal transplant patients, whereas only 12% to 16% of cytology samples display decoy cells.[11] In our study, BKV viruria was detected in 22.1% (207/938) of samples. Molecular studies can adequately identify and quantify viral material even in the presence of marked cellular degeneration and can be performed successfully in samples that have been stored for several days.[4]

A determination of viremia is not useful for screening because of the low sensitivity of the test.[4] Viremia is not present in patients with low-level/limited viral replication in the urinary tract. Increasing levels of viremia develop only if there is significant tissue damage with progression to BKVN. For this reason, quantitation of BKV viremia has emerged as the most specific non-

invasive test to confirm BKVN.[17]

In contrast, the BK viruria and viremia threshold associated with BKVN has not been defined. Because of the paucity of comparative studies on large numbers of renal transplant recipients, no widely accepted consensus regarding the use of urine cytology or PCR for screening purposes has emerged.[11] In one study regarding urine BKV load, it was suggested that repeated values of $> 10^7$ copies/mL are associated with BKVN, while the detection of BK viremia at $> 10^4$ copies/mL is considered the most reliable surrogate marker of presumed BKVN in renal transplant recipients.[20] However, in another report, a BKV load 1.6×10^4 copies/mL in blood (or 2.5×10^7 copies/mL in urine) was strongly associated with concurrent BKVN, and this finding supports the recent consensus for performing an allograft biopsy to diagnose BKVN in renal transplant recipients with a BKV load $> 1.0 \times 10^4$ copies/mL in blood.[7]

In our study, we incorporated urine and plasma standards for BKV DNA of 1.0×10^4 copies/mL into the quantitative assay.

A BKVN diagnosis is highly unlikely in the absence of BKV viruria or viremia by PCR. Hence, BKV PCR may be a clinically useful non-invasive test for identifying renal transplant recipients at high risk for concurrent BKVN. Moreover, like most studies investigating BKVN, our study was limited by the low number of BKVN cases. The identification of a diagnostic cut-off should require further studies, considering the potential sampling error and the evaluation of surrogate viral replication markers.

In conclusion, quantifying BKV in blood and urine is superior to urine cytology for concurrent BKVN. Considering that an early diagnosis of BKV infection with a reduction of immunosuppression could reverse viremia and retard progression of BKVN, BKV screening by quantitative RT-PCR assays may be a clinically useful non-invasive test for detecting concurrent BKVN in renal allograft recipients.

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