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Dual Positivity of Donor and Recipient Plasma for BK Virus Confers a High Risk for Development of BK Nephropathy in Renal Allograft

R. Thakur, K. Joshi, M. Minz, A. Singla, R. Nada, S. Arora, V. Jha, and V. Sakhuja

ABSTRACT

Background. BK nephropathy (BKN) is an important complication of renal transplantation, with a reported incidence between 1% and 10% in different parts of the world. Known risk factors for the development of BKN are the recently introduced immunosuppressants and steroids. However, the preexisting viral load may add to the risk for development of BKN. Therefore, the present study was designed to monitor the baseline BK virus (BKV) DNA in renal transplant donors and recipients in India for correlation with the development of BKN.

Methods. This study used real-time polymerase chain reaction (PCR) for quantification of BKV DNA in the plasma of kidney transplant donors (n = 38) and recipients (n = 87) at the time of surgery. The control BKV DNA was manufactured from a known positive human sample, by cloning a 133-bp PCR product of bases 4,329 to 4,462 of the large T-antigen (TAg) of BKV in a plasmid vector.

Results. Twenty-five of 87 recipient (28.7%) and 17/38 donor (44.7%) plasma samples were positive for BKV DNA at the time of transplantation with a median viral load of 910 (range 49–4770) and 312 (range 79–1508) copies per mL plasma, respectively. Six of 38 donor-recipient pairs showed viremia in both the recipient and donor: 1 developed histologically proven BKN at 18 months, 1 showed positive immunohistochemistry for SV40 TAg, and 2 others had high levels of viremia (14,545 copies at 6 and 2,617,524 copies at 3 months). None of the other 81 recipients showed evidence of BKN in the follow-up period.

Conclusions. This study showed that 28% of recipients and 44% of donors displayed baseline positivity for BKV DNA in plasma, which is higher than the reported incidence in the West. The baseline levels of BKV DNA in recipients with end-stage renal disease were higher than in donors. Dual positivity for BKV DNA in the plasma of donor-recipient pairs conferred a high risk of development of BK nephropathy in the allografted kidney.

BK nephropathy (BKN), an important complication of renal transplantation, was first recognized in 1995.[1] It affects 1%–10% of renal transplant recipients in different parts of the globe.[2–5] We first reported BK nephropathy in India with a seemingly high incidence of 9.3% among our renal transplant patients.[4] Polyomavirus-BK is a ubiquitous virus that shows a seroprevalence of 65%–90% in various populations by the age of 10 years.[6] However, not all renal transplant recipients who show decoy cells or BK virus (BKV) DNA in plasma or urine develop BKN. The virus remains latent; the factors leading to its reactivation or disease production remain largely unknown. The dynamics of reactivation and multiplication to sufficient numbers of latent BKV to produce BKN are complex and not fully understood. Highly effective immunosuppression, steroid treatment, and acute rejection episodes causing tubular

From the Departments of Histopathology (R.T., K.J., R.N.), Transplant Surgery (M.M.), Immunopathology (A.S., S.A.), and Nephrology (V.S., V.J.), Postgraduate Institute of Medical Education and Research, Chandigarh, India.

Address reprint requests to Dr Kusum Joshi, Department of Histopathology, Postgraduate Institute of Medical Education & Research, Chandigarh, India. E-mail: kus_joshi@yahoo.com

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damage are postulated to be risk factors for the development of BKN. Further more, the disease may be produced in recipients by the virus existing in the dormant state in the donor kidney or in the recipient's native kidneys and homing to the donor organ after reactivation. The 2 mechanisms are not mutually exclusive. Thus, preexistent viral load in either the donor or the recipient may add to the risk of development of BKN in the renal transplant.

The present study sought to quantify baseline BKV DNA load in donor and recipient plasma from kidney transplantations, using the sensitive molecular technique of real-time polymerase chain reaction (PCR). The control BKV DNA was a 133-bp cloned product of bases 4,329 to 4,462 of the large T-antigen of BKV. The recipients were followed after transplantation for development of BKN or high numbers of BKV DNA copies in plasma.

MATERIALS AND METHODS

Study Design

This prospective study on renal transplant recipients and live (except one, a deceased donor) donors was performed from January 2005 to December 2007. The 87 recipient plasma samples obtained within 24 hours after surgery and 38 donor plasma samples were analyzed by quantification of plasma BKV DNA by real-time PCR. Our Human Studies Ethics Committee approved the study and each of the donors and recipients gave informed consent before inclusion in the study.

Quantitative Measurement of Plasma BKV DNA

Standard positive control to quantify plasma BKV DNA was manufactured by cloning a 133-bp PCR product of known BKV-positive human DNA a including bases 4,329 to 4,462 of the large T-antigen of BKV in a plasmid vector pTZ57R/T using the Instant cloning kit (Fermentas Life Sciences, cat no. K1214), as described earlier by Thakur et al.[10] Successful ligation was confirmed by sequencing the plasmid. Plasmid concentrations were measured using a standard curve from 10 to 10^6 BKV copies per 5 μL DNA.[7]

DNA was extracted from 200 μL plasma samples with the QIAamp DNA blood mini kit (Qiagen, cat no. 51106) using a DNA filtration vacuum protocol yielding a final extraction volume of 50 μL. Sybr Green chemistry was used to amplify DNA in the samples. The forward primer 5′-AGCAGGCAAGGGTTCTATTACTA-AAT-3′ and reverse primer 5′-AAGCAACAGCAGATTCT-CAACA-3′ were specific for the large T-antigen of BKV genome.[8]

Quantitative PCR amplification reactions were run in a reaction volume of 25 μL containing 5 μL template DNA, 12.5 μL 1× final concentration of Maxima Sybr Green qPCR master mix (Fermentas Life Sciences, cat no. K0221), and 20 pmol each of forward and reverse primers. Thermal cycling was initiated by denaturation (10 min at 95°C) followed by 42 cycles of 30 s at 95°C, 32 s at 52°C, and 32 s at 72°C. Thereafter, the fluorescence was read. Contamination of the DNA was examined by melting-curve analysis on the PCR products from each run. Real-time PCR amplification data were analyzed with software provided by the manufacturer. The number of BKV copies of viral DNA per mL plasma was calculated from the standard curve. A negative control consisting of PCR-grade water and a positive DNA control was included in each run.[7]

All assays regarding quantification of BKV DNA were repeated by reextraction of DNA from stored plasma samples from the renal donors and recipients using the same extraction kit and procedures. Quantitative PCR experiments were repeated to determine the BKV copies in all plasma samples. All of the previously BKV DNA–positive plasma samples were found to be positive upon repeat analysis. A mean of the 2 values was taken as the BKV copy load per mL plasma.

RESULTS

Quantification of Plasma BKV DNA

The plasma samples of 87 recipients and 38 renal transplant donors were negative for BKV DNA by end-point PCR. However, 25/87 recipient samples (28.7%) showed detectable levels of BKV DNA by quantitative real-time PCR. Their BKV load ranged from 49 to 4,770 copies, with a median value of 910 copies per mL plasma (Table 1). Among the 25 plasma PCR-positive recipients, 24 were male aged 17–50 years (mean 33) and only 1 was female, aged 35 years.

Among the donor plasma samples, 17/38 (44.7%) showed detectable levels of BKV DNA. The BKV copies per mL plasma in the healthy donor population ranged from 79 to 1,508 BKV copies, with a median viral load of 312 (Table 1). Out of 17 plasma PCR-positive donors, 9 (53%) were female aged 19–50 years (mean 37) and 8 (47%) were male aged 26–57 years (mean 46).

Although the viremia prevalence in donor was higher than in recipients, the latter showed a higher median virus load; 910 vs 312 BKV copies per mL plasma. (Mann-Whitney test: $P = .017$). Also, 13.8% of recipients and only 2.6% of donors displayed BKV DNA values >1,000 copies per mL plasma.

Among 38 donor-recipient pairs tested at the time of transplantation, 6 showed detectable viremia in both samples. Their BKV copies outcomes are presented in Table 2 The recipient in pair no. 1 had 14,545 BKV copies per mL plasma at 6 months. The recipient in pair no. 2 developed histologically proven BK nephropathy at 18 months after transplantation. The recipient in pair no. 3 displayed 2,617,524 BKV copies at 3 months, but no biopsy was obtained and the patient was lost to follow-up. The recipient in pair no. 4 developed graft dysfunction at 8 days; the biopsy was unremarkable except for positive immunohistochemistry for SV40 large T-antigen. The recipients in pairs 5 and 6 experienced uneventful courses. Follow-up was available for the majority of the other 81 patients, who did not develop evidence of BKV activation or nephropathy from 6 months to 5 years.

Table 1. Values of Plasma BKV Copies in Renal Transplant Recipients and Donors at the Time of Transplantation

BKV Copies per mL Plasma	No. of Recipients	Number of Donors
Undetected	62 (71.27%)	21 (55.27%)
Detected	25 (28.73%)	17 (44.73%)
≤1,000	13 (14.94%)	16 (42.10%)
1,001–5,000	12 (13.79%)	1 (2.63%)
>5,000	0	0