

Prospective Study of BK Virus Infection and Nephropathy During the First Year After Kidney Transplantation

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Introduction. The aim of this study was to assess the prevalence and severity of BK virus infection, BK virus nephritis, and related risk factors among kidney transplant recipients.

Materials and Methods. BK viremia during the first year of kidney transplantation was assessed prospectively in 32 successive recipients. BK virus DNA was extracted and determined in all samples by real-time polymerase reaction assay for 1 year after kidney transplantation.

Results. The mean age of the patients was 33.3 ± 15.3 years. Sixteen patients (50%) received antithymocyte globulin for induction therapy. Living donor transplant consisted of 75% of the kidney donations. Maintenance immunosuppressive therapy included cyclosporine A in 27 patients (84.4%), plus tapering prednisolone and mycophenolate mofetil. BK viremia was detected in 8 patients (25%). The highest detected plasma viral load was less than 4000 copies per milliliter. BK virus was respectively positive in 5 (62.5%), 2 (25%), and 1 (12.5%) patients during the first 4, 8, and 12 months after transplantation. Biopsy-proven rejection and antirejection therapy by methylprednisolone pulses were 5 and 2.3 times more common in patients with BK virus infection ($P = .01$ and $P = .01$), respectively.

Conclusions. Despite occurrence of BK virus infection in 25% of our patients, BK nephropathy did not develop in any of them. Routine screening of BK virus infection, particularly in centers with low prevalence of BK virus nephritis, may not be cost effective for predicting this disease.

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INTRODUCTION

BK virus nephropathy (BKN) has been recognized as an emerging cause of allograft dysfunction in kidney transplant recipients.¹⁻³ It is postulated that after primary infection in childhood, the virus maintains its latency in uroepithelial cells.⁴⁻⁶ After kidney transplantation, reactivation of the virus can lead to BKN with definite diagnosis based on demonstration of characteristic pathologic changes

in renal biopsy samples.^{7,8} Frequency of BKN reportedly is 1% to 10%, with graft loss in up to 50% to 80% of cases if delayed diagnosis has been made.⁹⁻¹¹ Effective screening of BK virus in urine or plasma can tend to early detection of BKN, so that with early reduction of immunosuppression, the rate of graft loss may be reduced to 10% or less.¹⁰⁻¹⁸ Several studies have demonstrated that BK viremia appears a few weeks to months before

biopsy-proven diagnosis of BKN.¹⁹⁻²³

Although the reasons of recent increase in BK virus infection and nephritis remain mainly unknown, but increased knowledge of the physicians and also availability of more precise tests and monitoring for diagnosis exert some roles.²⁴ Increased overall immunosuppression by antirejection therapy with methyl prednisolone pulses and lymphocyte-depleting agents¹⁴ and use of tacrolimus and mycophenolate mofetil as maintenance therapy are well-known factors for uprising occurrence of this infection^{7,9,10,14,24-35}; however, BK virus infection has reported in patients on cyclosporine, azathioprine, or sirolimus therapies.³⁶⁻³⁸ In explaining the pathogenesis of BK virus infection that leads to BKN, the role of recipient humoral and cellular immunity,³⁹⁻⁴² allo-immune activation,⁴³ ischemic injury,¹⁴ and viral virulence^{44,45} should not be ignored.

In this longitudinal prospective study of kidney transplant recipients, we intended to assess the prevalence and severity of BK virus infection, BK virus nephritis, and related risk factors in our center.

MATERIALS AND METHODS

Participants

Thirty-two consecutive kidney transplant recipients at Shariati Hospital who signed the informed consent (between April 2010 and February 2011) were enrolled for blood sampling. They were prospectively followed up during the first year posttransplantation. In our center allograft biopsy is usually taken whenever plasma creatinine level increases more than 25% from baseline. The researchers were not aware of plasma viral load results during the follow-up.

Deceased kidney donor, second transplantation, young patients with a body weight less than 30 kg, and delayed graft function were indications for antithymocyte globulin induction therapy. Maintenance immunosuppression consisted of tapering prednisone, mycophenolate mofetil, and cyclosporine (except in 5 patients that received tacrolimus).

DNA Extraction

Blood samples of patients were taken during the first 4 (2 samples), 8, and 12 months posttransplantation. Plasma samples obtaining after blood centrifugation were kept at -80°C until

extraction. BK DNA was extracted from 200 µL of each patient's serum, using a QIA mp DNA Blood mini kit (Qiagen, Hilden, Germany), according to manufacturer's instruction. Briefly, 20 µL of protease was added to 200 µL sera in a 1.5-mL tube. Then, 200 µL of AI buffer was added to each tube, vortexed, and incubated for 10 minutes at 56°C. For DNA precipitation, 200 µL of ethanol was added to the mixture and centrifuged for 1 minute. Components transferred to a collection tube contained filter tube. Trapped DNA was washed in two steps by AW1 and AW2 buffers to eliminate purities together with centrifugation after each step. Finally, after centrifugation, 50 µL of elution buffer was added and stored at -20°C.

Real-time Polymerase Chain Reaction Assay

BK virus DNA was determined in all samples by real-time polymerase chain reaction assay according to manufacturer's instruction (BK RG Kit, Novin Gene Co, Tehran, Iran). This test is used to determine the presence of BK virus DNA in patients' specimens. Detection of the virus in these specimens may be indicative of an active infection, as polymerase chain reaction detects the presence of the virus, and not the host's reaction to the virus. As polyomavirus BK DNA detection in plasma is associated with an increased risk of BKN in renal recipients, quantitative testing may indicate change in transplant BKN risk over time.

Statistical Analysis

Statistical analysis of data was done using the SPSS software (Statistical Package for the Social Sciences, version 17.0, SPSS Inc, Chicago, Ill, USA). Qualitative data were compared using the chi-square test and the Fisher exact test, and quantitative data, by the Student *t* test. *P* values less than .05 was considered significant.

RESULTS

The mean age of the patients was 33.3 ± 15.3 years (Table). Seventeen patients (53.1%) were male. Sixteen patients (50%) received antithymocyte globulin induction therapy, 8 of whom were recipients from deceased donor, 2 had a second transplantation, 4 had delayed graft function and slow graft function, and 3 patients were young (ages of 8, 13, and 15 years). None of these patients developed BK virus infection. One patient received

Characteristics of 32 Kidney Transplant Recipients With and without BK Viremia

Characteristic	Kidney Transplant Recipients		
	All	Negative BK Viremia	Positive BK Viremia
Number of patients	32	24 (75.0)	8 (25.0)
Sex			
Male	17 (53.1)	13 (54.2)	4 (50.0)
Female	15 (46.9)	11 (45.8)	4 (50.0)
Mean age, y	33.3 ± 15.3	34.5 ± 17.0	29.8 ± 7.9
Donor source			
Living related	5 (15.6)	3 (12.5)	2 (25.0)
Living unrelated	19 (59.4)	14 (58.3)	5 (62.5)
Deceased	8 (25)	7 (29.2)	1 (12.5)
Induction therapy			
Antithymocyte globulin	16 (50)	13 (54.2)	3 (37.5)
Basiliximab	2 (6.3)	1 (4.2)	1 (12.5)
Maintenance therapy			
Cyclosporine A	27 (84.4)	20 (83.7)	7 (87.5)
Tacrolimus	5 (15.6)	4 (16.7)	1 (12.5)
Delayed graft function	4 (12.5)	3 (12.5)	1 (12.5)
Methyl prednisolone therapy	16 (50)	9 (37.5)	7 (87.5)
Serum creatinine, mg/dL			
1st month	1.21 ± 0.17 (0.80 to 1.50)	1.19 ± 0.18 (0.80 to 1.50)	1.25 ± 0.14 (1.10 to 1.50)
4th month	1.26 ± 0.25 (0.70 to 2.10)	1.26 ± 0.26 (0.70 to 2.10)	1.26 ± 0.18 (0.93 to 1.50)
8th month	1.25 ± 0.24 (0.50 to 1.68)	1.25 ± 0.25 (0.50 to 1.68)	1.24 ± 0.18 (1.05 to 1.54)
12th month	1.35 ± 0.32 (0.50 to 2.00)	1.31 ± 0.31 (0.50 to 1.80)	1.44 ± 0.36 (1.06 to 2.00)

antithymocyte globulin induction for both delayed graft function and deceased donation. Living donor transplant consisted of 75% of the kidney donations. Maintenance immunosuppressive therapy included cyclosporine A in 27 patients (84.4%).

BK viremia was detected in 8 patients (25%). Three of 4 samples in 2 patients (25%), 2 of 4 samples in 1 patient (12.5%), and 1 of 4 samples in 5 patients (62.5%) were positive. The mean plasma viral load was 567 ± 119 copies per milliliter. The highest detected plasma viral load was less than 4400 copies. BK virus was respectively positive in 5 (62.5%), 2 (25%), and 1 (12.5%) patient during the first 4, 8, and 12 months after transplantation. BK virus infection developed within the first 4 months in 62.5% of the patients.

During the follow-up period, kidney transplant biopsy was performed in 15 recipients. The reported pathologic results were T-cell mediated rejection in 5 patients (2 patients, class IA; 3 patients, class IB); in 3 patients, borderline changes; in 6 patients, no significant changes; and in 1 patient, calcineurin inhibitor toxicity. Therefore, biopsy-proven rejection was seen in 8 patients (25%), most of which were mild rejection (borderline and T-cell mediated rejection class I) and responsive

to methylprednisolone pulse therapy (usually 0.5 g/d to 1 g/d for 3 consecutive days) and only 1 patient received anti-thymocyte globulin antirejection therapy. Of note, not considering borderline changes, the rate of rejection was 15%.

Methylprednisolone therapy was 2.3-fold more common in patients with BK virus infection ($P = .01$). Overall, 16 patients (50%), of whom 7 (22%) in the first 2 weeks and 9 (28%) during the first year after transplantation received methylprednisolone pulses for clinical or biopsy-proven rejection (2 patients received methylprednisolone pulses in both periods).

Of 8 patients with BK virus infection, 7 (87.5%) received methylprednisolone pulses for clinical or biopsy-proven rejection. In 6 patients, kidney transplant biopsy was performed. The pathology results were borderline changes in 2, cell-mediated rejection class IA in 2, cell-mediated rejection class IB in 1, and no significant changes in 1 patient. Biopsy-proven rejection was 5-fold (3 of 24 in BK virus infection-negative group and 5 of 8 in BK virus infection-positive group) more common in patients with BK virus infection ($P = .01$).

BK virus nephritis changes using periodic acid-Schiff and SV40 antigen staining were reported

in none of the biopsy samples. The mean serum creatinine levels in all quarterly samplings were not different between the two groups (BK infection positive versus negative). There was no significant difference in other parameters between the patients with and without BK virus infection.

DISCUSSION

This prospective single-center study was designed to evaluate active BK virus infection during the first year after kidney transplantation. Similar studies were carried out elsewhere, but characteristics of our patients were somewhat different. Living donation is dominant and rate of delayed graft function is low. Choice of calcineurin inhibitor is mostly cyclosporine, which poses weaker immunosuppression properties. Therefore, it was prudent to assess the incidence of BK virus infection, its predictive value for BKN, and the need for routine screening of BK virus after kidney transplantation in our different recipients.

The prevalence of BK viremia based on real-time polymerase chain reaction results was 25% (8 of 32 patients), which is between 13% and 29% reported in other prospective studies.^{14,46} These differences would be owing to the number of samplings and sampling time points, sensitivity of measurements, and basically different patients. BK virus infection developed within the first 4 months in 62.5% of the patients. Likewise, in other reports, the highest BK virus replication occurred within 3 to 6 months posttransplantation during the strongest immunosuppressive period.^{14,46,47} In our study, the mean viral load was 567 copies per milliliter, which is much lower than other reports.

Although real-time polymerase chain reaction is the method of choice for detection of BK virus, various assays differ significantly in specimen types, DNA extraction method, polymerase chain reaction primers and probes, and reference materials utilized to generate a standard curve. These differences can change the specificity, accuracy, and dynamic ranges of various real-time assays.⁴⁸

Despite the high BK virus infection rate, none of the recipients developed BKN. Although transplant biopsy was not routinely performed in all patients, and subtle nephritis could not be ruled out, serum creatinine levels were not different between those with and without BK virus infection. We know that even in recipients whose renal biopsy was done,

focal nature of renal involvement, particularly in the early courses of the nephritis, could lead to nondiagnostic renal biopsy,⁴⁹ and diagnosis of BKN may be missed in 25% to 37% of biopsy specimens only comprising of one small core of cortex.⁵⁰ In addition, none of the viral load values reached the recommended cutoff value of greater than 7000 or 10 000 copies per milliliter of plasma^{14,16} for BKN. Nonetheless, viral load cutoff value for BKN differs largely among laboratories, considering there are compelling differences in BK virus assay sensitivity and viral load values (even 10-fold difference).^{16,51} Nephritis can be seen with BK virus DNAemia less than 7000 copies per milliliter of plasma.^{16,24,52} Furthermore, positive predictive value of quantitative viremia for BKN is 50% (and in case of persistent viremia more than 10⁴ copies can reach 80%) and its negative predictive value is 100%.^{14,16,50,52} Of note, as previously reported, the prevalence of BKN in our center is low (< 1%).³⁸

The rate of acute rejection and antirejection therapy with methylprednisolone pulses exert a significant role for BK virus replication, as some studies with higher rejection episodes have reported more replication.¹⁴ We observed that patients with BK virus infection had higher rejection episodes (5-fold) and they received more methylprednisolone pulse (2.3-fold) for treatment of clinical or biopsy-proven rejection. Among 8 patients with BK virus infection, 7 (87.5%) received methylprednisolone pulses for clinical or biopsy-proven (5 cases) rejection.

Overall, in our patients, biopsy-proven rejection was 25% (usually mild with borderline changes or class I T-cell mediated rejection), and 50% of the recipients received methylprednisolone pulses during the first year posttransplantation. Our results revealed that in 5 recipients (62.5%) only 1 sample was positive for BK virus; therefore, BK virus infection was mostly transient and related to antirejection treatment. In agreement with our study, the reported incidence of BK viremia and BK viremia is 35% to 57% and 7% to 29%, respectively,^{14,17,46,53,54} and nearly 50% of the viremic episodes are transient (one-time event).^{17,53}

In a prospective study by Bressollette and colleagues⁴⁶ on 104 recipients who received anti-interleukin-2R (41.5%) and antithymocyte globulin (58.5%) induction therapy and were on tacrolimus (58.5%) as calcineurin inhibitor maintenance

therapy, viremia occurred in 29% of the recipients in the first year posttransplantation, and the risk for viremia was higher in patients treated with tacrolimus. The median plasma viral load reached 4.8×10^3 copies per milliliter and none developed BKN. They concluded that interruption of corticosteroid therapy in all of their patients before the third month posttransplantation and the low incidence of rejection (6%) may explain the absence of BKN development in their series. Likewise, in a prospective report by Hirsch and colleagues¹⁴ on 78 patients who received antithymocyte globulin and anti-interleukin-2R induction therapy in 6.5% and 10% of cases respectively and were on tacrolimus maintenance therapy (47%), viremia was detected in 13% of recipients in the first year posttransplantation and the rate of BKN was 6.5%. In their series, the rate of rejection and antirejection therapy by antithymocyte globulin (26%) and methylprednisolone pulses (38%) was relatively high and antirejection treatment, particularly with corticosteroids, was associated with BK virus replication and nephropathy. The viral load in plasma was higher in patients with BKN than in those without nephropathy (mean of 28 000 versus 2000 copies per milliliter, respectively).

In our series, only 5 recipients (15.6%) received tacrolimus as calcineurin inhibitor, one of which revealed BK virus infection. Likewise, induction by antithymocyte globulin (54.2% in BK virus-negative versus 37.5% in BK virus-positive recipients) and delayed graft function did not affect the rate of viremia, although the rate of delayed graft function was low (12.5%) in our recipients.

CONCLUSIONS

Routine screening of BK virus infection, particularly in centers with low prevalence of BKN, may not be cost effective (especially in developing countries) for predicting this disease. However, although routine screening of BK virus infection may not be done due to financial shortage (based on literature), it is extremely recommended for kidney dysfunction, after antirejection therapy, and to monitor clinical course of viral nephropathy.

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CONFLICT OF INTEREST

None declared.

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