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Impact of Combined Acute Rejection on BK Virus-Associated Nephropathy in Kidney Transplantation

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BK virus-associated nephropathy (BKVAN) is one of the major causes of allograft dysfunction in kidney transplant (KT) patients. We compared BKVAN combined with acute rejection (BKVAN/AR) with BKVAN alone in KT patients. We retrospectively analyzed biopsy-proven BKVAN in KT patients from 2000 to 2011 at Seoul National University Hospital. Among 414 biopsies from 951 patients, biopsy-proven BKVAN was found in 14 patients. Nine patients had BKVAN alone, while 5 patients had both BKVAN and acute cellular rejection. BKVAN in the BKVAN alone group was detected later than in BKVAN/AR group (21.77 vs 6.39 months after transplantation, $P = 0.03$). Serum creatinine at diagnosis was similar (2.09 vs 2.00 mg/dL). Histological grade was more advanced in the BKVAN/AR group ($P = 0.034$). Serum load of BKV, dose of immunosuppressants, and tacrolimus level showed a higher tendency in the BKVAN alone group; however it was not statistically significant. After anti-rejection therapy, immunosuppression was reduced in the BKVAN/AR group. Renal functional deterioration over 1 yr after BKVAN diagnosis was similar between the two groups ($P = 0.665$). These findings suggest that the prognosis of BKVAN/AR after anti-rejection therapy followed by anti-BKV therapy might be similar to that of BKVAN alone after anti-BKV therapy.

Key Words: Acute Rejection; BK Virus; Kidney Diseases; Kidney Transplantation

INTRODUCTION

BK virus (BKV) causes a nephropathy in kidney transplant recipients that had potent immunosuppressive drugs such as tacrolimus and mycophenolate mofetil (MMF). BK virus-associated nephropathy (BKVAN) is found in 1%-10% of kidney transplant patients, and 50%-60% of them resulted in allograft loss or permanent dysfunction (1, 2). Diagnosis for BKVAN is based on histological findings characterized by lymphocytic interstitial infiltrates and the nuclear reaction to the anti-SV-40T antibody as evidence of viral replication, and positive polymerase chain reaction for BKV DNA. However, it is difficult to make a clear differential diagnosis between BKVAN and acute cellular rejection, because pathologic features of the two entities are overlapped (3). Moreover, both might coexist at the same time, and the relationship between their cause and effect remain controversial (4).

The main treatment for BKVAN is reduction of maintenance immunosuppression (5). Conversion of tacrolimus to cyclosporine, reduction of calcineurin inhibitors or antiproliferative agents, conversion of MMF to leflunomide, and discontinuation of MMF have been used for treatment of BKVAN, with monitoring of serum PCR titer of BKV. Recently, conversion of calcineurin inhibitors to sirolimus or everolimus is also suggested (6). Other treatment options are antiviral treatment such as cidofovir, and intravenous immunoglobulin, which might be especially considered in the case of simultaneous BKVAN and acute cellular rejection (5, 7). In fact, treatment for BKVAN superimposed on acute cellular rejection is not established, and prognosis of this situation is not known.

Here, we compared BKVAN combined with acute rejection with BKVAN alone in kidney transplant patients, and investigated the risk factors and prognosis of both conditions.

MATERIALS AND METHODS

Between January 2000 and December 2011, 951 patients received kidney transplants at Seoul National University Hospital in Seoul, Republic of Korea. We collected cases of biopsy-proven BKVAN and their data about age, sex, etiology of original renal disease, donor type, episodes of rejection and infection, diagnosis time of BKVAN from transplantation, comorbid conditions, immunosuppressive and antiviral therapy, BK viral load, and clinical outcomes such as estimated glomerular filtration

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rate (eGFR), graft loss, and death. BK viral load was measured using real time PCR techniques for serum samples, whenever renal function deterioration was observed. The eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) formula (8). Renal functional changes over 3 months and 12 months after BKVAN diagnosis were compared between the BKVAN alone group and the group of BKVAN combined with acute rejection (BKVAN/AR).

Differential diagnosis between BKVAN alone and BKVAN/AR was made according to histopathological features (2). When interstitial infiltration of inflammatory cells and tubulitis was mainly detected in the area of $SV40^+$ in immunohistochemical staining, the cases were categorized to BKVAN alone. On the other hand, cases where interstitial inflammation was scattered to $SV40^-$ area, were categorized to BKVAN/AR. Pathological grading was also performed according to UMD/AST classification (9), and scoring system of 2007 Banff (10, 11).

We compared baseline characteristics, clinical features at the time of BKVAN, and outcomes between the two groups. A chi-square test was used for analyzing categorical variables, and continuous variables were analyzed by a t-test. All statistical analyses were performed using the SPSS statistical software (version 18.0.0, SPSS Inc., Chicago, IL, USA), and $P < 0.05$ was considered statistically significant.

The study was approved by the institutional review board of Seoul National University Hospital (H-1212-064-450). Informed consent was waived by the IRB. The study was conducted under the Declaration of Helsinki guidelines for human subject research as well as the Declaration of Istanbul 2008.

RESULTS

A total of 14 cases with biopsy-proven BKVAN was identified, and it reached about 4% of the study population. Their baseline characteristics were summarized in Table 1. The mean age of the recipients was 44.57 ± 16.60 yr, and 4 patients were male. There was no case of retransplantation. Nine patients had BKVAN alone, while 5 patients had BKVAN/AR. Immunosuppressive regimens and frequency of rejection before BKVAN were similar between the two groups.

BKVAN/AR occurred earlier than BKVAN alone (6.39 ± 2.45 vs 21.77 ± 11.04 months after transplantation, $P = 0.003$). Renal function deteriorated in all patients by 20.44 ± 14.09 mL/min during the 3 months before diagnosis of BKVAN. However, renal functional loss was similar between the two groups ($P = 0.647$, Table 2). Serum load of BKV in the BKVAN alone group showed a higher tendency than the BKVAN/AR; however, it was not statistically significant. In parallel, both dose of prednisolone/MMF and trough levels of tacrolimus showed a higher tendency in BKV alone group, despite statistically insignificant (Table 2). When UMD/AST classification was applied, the BKVAN/AR group had higher proportion of B3 than the BKVAN alone group ($P = 0.034$, Table 2).

Treatment and outcomes were summarized in Table 3. In the BKVAN alone group, 3 patients switched tacrolimus to cyclosporine, and 6 patients reduced tacrolimus dose. MMF was switched to mizoribin and sirolimus in 2 and 1 patient, respectively. Four patients reduced MMF dose, and 1 patient quit MMF. Mean serum BKV load decreased from 1,163,210 to 2,200

Table 1. Baseline characteristics

Characters	BKVAN alone (n = 9)	BKVAN/AR (n = 5)	Total (n = 14)	*P* value*
Age (yr)	46.44 ± 15.12	41.20 ± 20.42	44.57 ± 16.60	0.592
Age at transplantation	41.60 ± 14.92	39.16 ± 19.65	40.73 ± 16.04	0.797
Male	3	1	4	0.280
Living donor	6	3	9	1.000
First graft	9	5	14	1.000
Etiology of ESRD				
Diabetes mellitus	1	0	1	
Glomeruonephritis	3	1	4	
Cystic disease	1	1	2	
Others or unknown	4	3	7	
Basiliximab induction	6	5	11	0.258
Maintenance immunosuppression				0.357
Triple (prednisolone + CNI + MMF)	9	4	13	
Double (prednisolone + CNI)	0	1	1	
History of acute rejection before BKVAN	3	2	5	1.000
Number of previous rejection	0.56 ± 0.73	0.60 ± 0.89	0.57 ± 0.76	0.921
Treatment of previous rejection				1.000
Steroid pulse	3	2	5	
OKT3/ATG	0	0	0	

Numerical values were expressed as the mean ± standard deviation. *The chi-square test was used for the categorical data. The numerical data were compared using a t-test as appropriate. BKVAN, BK virus nephropathy; BKVAN/AR, BK virus nephropathy combined with acute rejection; ESRD, end stage renal disease; CNI, calcineurin inhibitor; MMF, mycophenolate mofetil; ATG, thymoglobulin.

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Table 2. Clinical and pathological characteristics at the time of BKVAN

Parameters	BKVAN alone (n = 9)	BKVAN/AR (n = 5)	Total (n = 14)	*P* value*
Time from transplantation to BKVAN diagnosis (months)	21.77 ± 11.04	6.39 ± 2.45	16.28 ± 11.64	0.003
Baseline serum creatinine at 3 months before BKVAN diagnosis (mg/dL)	1.44 ± 0.42	1.42 ± 0.65	1.43 ± 0.48	0.938
Serum creatinine at BKVAN diagnosis (mg/dL)	2.09 ± 0.64	2.01 ± 0.65	2.06 ± 0.64	0.811
ΔeGFR during the last 3 months before BKVAN diagnosis (mL/min)	-19.08 ± 12.70	-22.88 ± 17.63	-20.44 ± 14.09	0.647
Concomitant viral infection	1	2	3	0.510
Initial load of serum BKV (copies/mL)	1,140,700 ± 1,525,395	276,708 ± 294,925	747,976 ± 1,183,974	0.248
Peak load of serum BKV (copies/mL)	1,163,210 ± 1,516,467	294,340 ± 299,757	768,269 ± 1,179,690	0.243
Dose of prednisolone at BKV diagnosis (mg/day)	6.11 ± 2.83	4.75 ± 1.85	5.63 ± 2.54	0.356
Blood trough level of tacrolimus at BKV diagnosis (ng/mL)	7.22 ± 1.08	6.46 ± 2.04	6.95 ± 1.46	0.475
Dose of mycophenolate mofetil at BKV diagnosis (mg/day)	1111.10 ± 469.55	750.00 ± 433.01	982.14 ± 474.99	0.183
B3 by UMD/AST pathological classification	1	4	5	0.034
t3 by 2007 Banff classification	1	3	4	0.095
i3 by 2007 Banff classification	4	2	6	1.000
t+i > 2 by 2007 Banff classification	8	5	13	1.000
ct+ci > 2 by 2007 Banff classification	1	1	2	1.000

Numerical values were expressed as the mean ± standard deviation. *The chi-square test was used for the categorical data. The numerical data were compared using a t-test as appropriate. BKVAN, BK virus nephropathy; BKVAN/AR, BK virus nephropathy combined with acute rejection; Δ, change; eGFR, estimated glomerular filtration rate.

Table 3. Treatment and outcomes of BKVAN

Outcomes	BKVAN alone (n = 9)	BKVAN/AR (n = 5)	Total (n = 14)	*P* value*
Follow-up duration (months)	23.11 ± 19.03	24.76 ± 15.67	23.74 ± 17.14	0.874
Treatment				
Steroid pulse	3	4	7	0.070
ATG	0	1	1	1.000
Tacrolimus reduction	6	3	9	1.000
Conversion of tacrolimus to cyclosporine A	3	1	4	1.000
MMF reduction	4	0	4	0.221
Discontinuation of MMF	1	0	1	1.000
Conversion of MMF to leflunomide	0	4	4	0.005
Conversion of MMF to sirolimus	1	0	1	1.000
Conversion of MMF to mizoribin	2	0	2	0.505
Intravenous immunoglobulin	0	2	2	0.128
ΔeGFR during 3 months after treatment (mL/min)	6.43 ± 6.46	-0.72 ± 11.19	4.48 ± 8.82	0.150
ΔeGFR during 1 yr after treatment (mL/min)	2.53 ± 8.02	-0.77 ± 16.06	2.60 ± 10.14	0.665
Load of serum BKV at 1 yr after treatment (copies/mL)	2,200 ± 1,642	50 ± 71	1,483 ± 1,689	0.156
Rejection after BKVAN	2	1	3	1.000
Graft loss	2	1	2	1.000
Patient death	1	0	1	1.000

Numerical values were expressed as the mean ± standard deviation. *The chi-square test was used for the categorical data. The numerical data were compared using a t-test as appropriate. BKVAN, BK virus nephropathy; BKVAN/AR, BK virus nephropathy combined with acute rejection; ATG, thymoglobulin; MMF, mycophenolate mofetil; Δ, change; eGFR, estimated glomerular filtration rate.

copies/mL over 1 yr after diagnosis, and renal function was stabilized during 1 yr after treatment (ΔeGFR during 1 yr after treatment, 2.52 ± 8.01 mL/min). Rejection occurred in 2 cases after reduction of immunosuppression. Two patients lost renal allograft; however, the causes of graft failure were not related to BKVAN (recurrent IgA nephropathy and transitional carcinoma of renal pelvis). One patient died of heart failure.

In the BKVAN/AR group, 4 patients were treated with steroid pulse, and ATG was administered in 1 patient. After anti-rejection therapy, one patient switched tacrolimus to cyclosporine, and 3 patients reduced tacrolimus dose. Four patients switched MMF to leflunomide, and 2 patients received intravenous immunoglobulin. Mean serum BKV load decreased from 294,340 to 50 copies/mL over 1 yr after diagnosis, and renal function was stabilized during 1 yr after treatment (ΔeGFR during 1 yr after treatment, -0.77 ± 16.06 mL/min). Rejection recurred in one case in the BKVAN/AR group, and 1 lost renal allograft.

When the BKVAN alone group was compared with the group of BKVAN combined with acute rejection, serum load of BKV, dose of immunosuppressants, and tacrolimus level showed a higher tendency in the BKVAN alone group; however it was not statistically significant (Table 3, Fig. 1B). Although renal function decreased before treatment, treatment stabilized renal function in both groups, and renal functions were similar between the two groups at all time-points (Fig. 1A). These data showed that reduction of immunosuppression with anti-rejec-

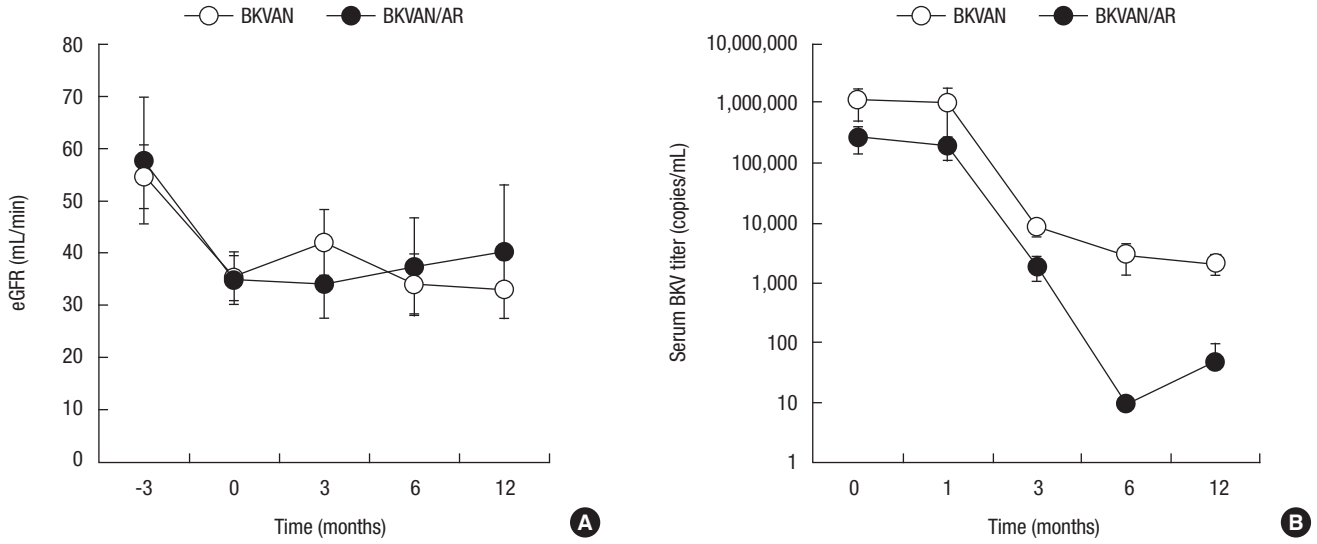

Fig. 1. Courses of renal function and BK viral load in BK virus nephropathy with or without acute rejection. (**A**) After abrupt reduction of renal function before treatment of BKVAN, renal function was stabilized over 1 yr after BKVAN. There was no significant difference in renal function in 1 yr after diagnosis of BKVAN between BKVAN alone and BKVAN combined with acute rejection. Zero time indicated diagnosis time for BKVAN. Each value was expressed as mean with standard error. $P > 0.05$ at all time points. (**B**) BK viral load had decreased after treatment of BKVAN in both groups. Although BK viral loads in the BKV alone group were higher than those in the BKVAN combined with acute rejection group, the differences were not statistically significant ($P > 0.05$ at all time-points). Zero time indicated diagnosis time for BKVAN. Each value was expressed as mean with standard error. eGFR, estimated glomerular filtration rate. BKVAN, BKVAN alone; BKVAN/AR, BKVAN combined with acute rejection.

tion therapy in BKVAN/AR led to comparable outcomes to those of BKVAN alone.

DISCUSSION

The incidence and the mean creatinine level at diagnosis of BKVAN in the present study were similar as previously reported. However, lack of protocol biopsy and empirical anti-rejection therapy must have underestimated the incidence of BKVAN.

In pathological diagnosis of BKVAN, it is not easy to morphologically distinguish acute cellular rejection without endarteritis or glomerulitis from BKVAN itself. Hirsch et al. (2) described that tubular HLA-DR expression, lymphocytic infiltrate, and marked tubulitis in areas lacking polyomavirus replication may support the diagnosis of concurrent cellular rejection. Based on this concept, we categorized BKVAN patients to 2 groups- BKVAN alone and BKVAN/AR.

When we compared the two groups, BKVAN/AR occurred in earlier than BKVAN alone, possibly because the risk for acute rejection is high within 1 yr after transplantation. Both strength of immunosuppression and serum titer of BKV was slightly higher in the BKVAN alone group, despite the difference was not statistically significant due to small sample size. Overimmunosuppression could suppress concurrent rejection sufficiently in the BKVAN alone group.

University of Maryland and American Society of Transplantation proposed histological grading system to BKVAN according to degree of tubular cytopathic injury, interstitial inflammation, or atrophy and fibrosis (9). According to the UMD/AST grading, the BKVAN/AR had higher grade than the BKVAN alone, and these results suggested that the BKVAN in the BKVAN/AR group was worse. However, it is more likely that interstitial inflammation resulting from acute cellular rejection, rather than the severity of BKVAN itself, contributed to the higher UMD/ATS grade in the BKVAN/AR group.

Reduction of calcineurin inhibitors and conversion of tacrolimus to cyclosporine is main treatment for BKVAN, because calcineurin inhibitor, especially tacrolimus suppressed anti-BKV T cell activity (12). Because sirolimus increased anti-BKV memory T cells in mice (13), and spare anti-BKV T cell activity in humans (12), switching MMF to sirolimus with reduction of tracrolimus is also suggested. However, reduction of immunosuppression alone might aggravate concurrent acute rejection in the BKVAN/AR group. Therefore, we administered anti-rejection therapy at first, and then reduced maintenance immunosuppression slowly in the BKVAN/AR group. By this protocol, the BKVAN/AR group stabilized renal function and achieved similar graft outcomes as the BKVAN alone group. However, one third of patients with BKVAN alone also received empirical steroid pulse therapy, and this factor might have contributed to the similar outcomes as those in BKVAN/AR group with anti-rejection therapy.

The present study has a few limitations. First, the small sample size and the retrospective design of the present study need further large-scaled, prospective, multicenter studies for validation of our results. Second, either protocol biopsy before treatment or follow-up biopsy after treatment was not performed routinely. Third, high variability of treatment regimens for BK-

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VAN and BKVAN/AR made it difficult to evaluate their treatment responses for both conditions.

In summary, we compared clinical features of BKVAN combined with acute cellular rejection and BKVAN alone, and demonstrated that the reduction of immunosuppression following anti-rejection therapy stabilized renal functional deterioration in BKVAN/AR, and that it led to similar outcomes as those in BKVAN alone.

DISCLOSURE

We declare that we have no conflicts of interest.

REFERENCES

1. Hirsch HH, Knowles W, Dickenmann M, Passweg J, Klimkait T, Mihatsch MJ, Steiger J. *Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. New Engl J Med 2002; 347: 488-96.*
2. Hirsch HH, Brennan DC, Drachenberg CB, Ginevri F, Gordon J, Limaye AP, Mihatsch MJ, Nickeleit V, Ramos E, Randhawa P, et al. *Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analyses and recommendations. Transplantation 2005; 79: 1277-86.*
3. Drachenberg CB, Hirsch HH, Ramos E, Papadimitriou JC. *Polyomavirus disease in renal transplantation: review of pathological findings and diagnostic methods. Hum Pathol 2005; 36: 1245-55.*
4. McGilvray ID, Lajoie G, Humar A, Cattral MS. *Polyomavirus infection and acute vascular rejection in a kidney allograft: coincidence or mimicry? Am J Transplant 2003; 3: 501-4.*
5. Ramos E, Drachenberg CB, Wali R, Hirsch HH. *The decade of polyomavirus BK-associated nephropathy: state of affairs. Transplantation 2009; 87: 621-30.*
6. Sánchez Fructuoso AI, Calvo N, Perez-Flores I, Valero R, Rodríguez-Sánchez B, García de Viedma D, Muñoz P, Barrientos A. *Mammalian target of rapamycin signal inhibitors could play a role in the treatment of BK polyomavirus nephritis in renal allograft recipients. Transpl Infect Dis 2011; 13: 584-91.*
7. Ito Y, Nishi S, Imai N, Yoshita K, Saito K, Nakagawa Y, Takahashi K, Narita I. *The case of BK virus infection in which it was difficult to differentiate from acute rejection. Clin Transplant 2011; 25: 44-8.*
8. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. *A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation: Modification of Diet in Renal Disease Study Group. Ann Intern Med 1999; 130: 461-70.*
9. Masutani K, Shapiro R, Basu A, Tan H, Wijkstrom M, Randhawa P. *The Banff 2009 Working Proposal for polyomavirus nephropathy: a critical evaluation of its utility as a determinant of clinical outcome. Am J Transplant 2012; 12: 907-18.*
10. Solez K, Colvin RB, Racusen LC, Haas M, Sis B, Mengel M, Halloran PF, Baldwin W, Banfi G, Collins AB, et al. *Banff 07 classification of renal allograft pathology: updates and future directions. Am J Transplant 2008; 8: 753-60.*
11. Hirsch HH, Randhawa P; AST Infectious Diseases Community of Practice. *BK virus in solid organ transplant recipients. Am J Transplant 2009; 9: S136-46.*
12. Egli A, Köhli S, Dickenmann M, Hirsch HH. *Inhibition of polyomavirus BK-specific T-cell responses by immunosuppressive drugs. Transplantation 2009; 88: 1161-8.*
13. Araki K, Turner AP, Shaffer VO, Ganqappa S, Keller SA, Bachmann MF, Larsen CP, Ahmed R. *mTOR regulates memory CD8 T-cell differentiation. Nature 2009; 460: 108-12.*