Introduction

BK virus-associated nephropathy (BKVAN) is an impor-
Methods

Study design

We retrospectively investigated the medical records of 582 patients that underwent KT between June 2001 and December 2014. We checked urine decoy cells to screen for BK virus infection every month for 12 months after KT and checked it regularly every year thereafter. Among 582 KTRs, 258 patients had urinary decoy cells and 324 patients did not. Allograft biopsies were performed when a positive urine decoy cell was detected [6] and allograft dysfunction or proteinuria occurred [7]. BKVAN was diagnosed by the pathologic confirmation of allograft biopsies and classified into stages A, B, and C according to Banff criteria 2009 [8]. “Class A” was defined as a variable number of virus-infected cells with no or minimal injury to tubular epithelial cells, “class B” was defined as tubular epithelial cell necrosis or lysis with denudation of the basement membrane across a length of more than 2 cells, and “class C” was defined as any degree of tubular injury with interstitial fibrosis that affected more than 50% of the cortex. Among 258 KTRs with positive urinary decoy cells, allograft kidney biopsy was performed in 47 patients, and 15 KTRs were diagnosed with BKVAN based on allograft biopsy indications. Because the urine decoy cell test had a 100% negative predictive value [9], we divided the diagnoses into a BKVAN group (15 patients) diagnosed by allograft kidney biopsies and a control group (356 patients), with the exception of 211 patients that did not undergo allograft biopsies with urine decoy cells (Fig. 1). Allograft function was measured as serum creatinine by the Jaffe method and an estimated glomerular filtration rate (eGFR) by the Modification of Diet in Renal Disease (MDRD) formula before the diagnosis, at the time of diagnosis, and 12 months after the diagnosis of BKVAN [10]. Allograft dysfunction was defined as an increase of more than 30% of serum creatinine level or decrease of more than 50% of eGFR compared to baseline allograft function. Acute rejection was classified as borderline, cellular rejection, and antibody-mediated rejection through the allograft kidney biopsies. Graft failure was defined as a return to dialysis or re-transplantation. The treatment options varied; discontinuation or reduction of mycophenolate mofetil (MMF) and reduction or substitution of tacrolimus with cyclosporine in addition to sirolimus, leflunomide, or fluoroquinolone were performed to treat BKVAN. We performed follow-up allograft kidney biopsies at two months after treatment of BKVAN with acute rejection.

In the subgroup analysis, we divided the KTRs who underwent allograft kidney biopsies into four groups as follows: With or without BKVAN and with or without acute rejection. We evaluated the long-term outcomes of BKVAN and the clinical significance of BKVAN on the post-transplant clinical outcomes.

This study was approved by the Institutional Review Board of Keimyung University School of Medicine (2016-11-047).

Statistical analyses

We analyzed the data using Student’s t test or Mann-Whitney test for continuous variables and the chi-square test and Fisher’s exact test for categorical variables. Continuous variables were presented as means ± standard deviation.
deviations or medians (interquartile ranges [IQRs]) and
categorical variables as numbers and proportions (%). P
values < 0.05 were considered statistically significant.
Statistical analysis was performed using PASW Statistics
ver. 18.0 (IBM Co., Armonk, NY, USA).

Results

Baseline characteristics of BK virus-associated nephropathy

The incidence of BKVAN was 4.0% (15 of 371 KTRs) dur-
ing the study period. Follow-up duration was 93.1 ± 52.3
months. The median time from KT to BKVAN diagnosis
was 5.9 (IQR, 4.4–8.7) months, and the follow-up dura-
tion after BKVAN diagnosis was 58.5 (IQR, 32.2–123.4)
months. The median (IQR) age of the KTRs at BKVAN
diagnosis was 50 (40–53) years, and 13 (86.7%) were
men. Six patients (40.0%) underwent living donor KTs,
and 14 (93.3%) patients experienced their first KT. Eleven
patients (73.3%) received hemodialysis before KT, and
11 (73.3%) had chronic glomerulonephritis as a cause of
end-stage renal disease. Tacrolimus was used in all pa-
tients, and the median (IQR) trough level of tacrolimus
was 9.1 (5.9–12.2) ng/mL at diagnosis of BKVAN. Ten
patients (66.7%) had biopsy-proven acute rejection. The
median (IQR) serum creatinine level and eGFR at diag-
nosis were 2.3 (2.0–3.0) mg/dL and 29.2 (20.3–35.3) mL/
min/1.73 m², respectively. According to the Banff 2009
Working Proposal, 6 (40.0%) patients were stratified as
stage A, 8 (53.3%) as stage B, and 1 (6.7%) as stage C.

Comparison of baseline characteristics between BKVAN
group and control group

We compared the clinical and laboratory parameters
between the BKVAN group and control group (Table 1).
The proportion of male recipients was significantly high-
er in the BKVAN group compared with the control group

| Variable                        | BKVAN group (n = 15) | Control group (n = 356) | P value |
|--------------------------------|----------------------|-------------------------|---------|
| Recipient age (yr)              | 47.9 ± 9.1           | 42.4 ± 11.7             | 0.074   |
| Recipient male gender           | 13 (86.7)            | 198 (55.6)              | 0.017   |
| Donor age (yr)                  | 46.8 ± 5.6           | 39.7 ± 13.1             | < 0.001 |
| Donor male gender               | 6 (40.0)             | 205 (57.6)              | 0.287   |
| Donor type                      |                      |                         | 0.179   |
| Living; deceased                | 6 (40):9 (60)        | 213 (59.8):143 (40.2)   | 1.000   |
| KT number                       | 14 (93.3):1 (6.7)    | 309 (86.8):47 (13.2)    | 0.336   |
| Dialysis type before KT         |                      |                         |         |
| Hemo dialysis: Peritoneal dialysis | 11 (73.3):4 (26.7) | 259 (72.8):59 (16.6) | 0.385   |
| Cause of end-stage renal disease|                      |                         |         |
| Glomerulonephritis              | 11 (73.3)            | 268 (75.3)              |         |
| Diabetes mellitus               | 1 (6.7)              | 50 (14.0)               |         |
| Hypertension                    | 1 (6.7)              | 15 (4.2)                |         |
| ADPKD                           | 1 (6.7)              | 11 (3.1)                |         |
| Others                          | 1 (6.7)              | 12 (3.4)                |         |
| HLA mismatch number             | 4.3 ± 1.7            | 3.3 ± 1.6               | 0.053   |
| Induction immunosuppressant     |                      |                         | < 0.001 |
| Basiliximab                     | 10 (66.7)            | 240 (67.4)              |         |
| Antithymocyte globulin          | 2 (13.3)             | 15 (4.2)                |         |
| None                            | 3 (20)               | 101 (28.4)              |         |
| Main immunosuppressant          |                      |                         | 0.611   |
| Tacrolimus                      | 15 (100)             | 332 (93.3)              |         |
| Cyclosporine                    | 0                    | 24 (6.7)                |         |

Data are expressed as the mean ± standard deviation or number (%).
ADPKD, autosomal dominant polycystic kidney disease; BKVAN, BK virus-associated nephropathy; HLA, human leukocyte antigen; KT, kidney transplantation.
(86.7% vs. 55.6%, \( P = 0.017 \)). The mean age of recipients in the BKVAN group tended to be older than in the control group (47.9 ± 9.1 vs. 42.4 ± 11.7, \( P = 0.074 \)). The mean age of kidney donors was significantly higher in the BKVAN group compared with the control group (46.8 ± 5.6 vs. 39.7 ± 13.1, \( P < 0.001 \)). The number of human leukocyte antigen (HLA) mismatches tended to be higher in the BKVAN group compared with the control group (4.3 ± 1.7 vs. 3.3 ± 1.6, \( P = 0.053 \)). The proportion of antithymocyte globulin as an induction immunosuppressant was significantly higher in the BKVAN group compared with the control group (13.3% vs. 4.2%, \( P = 0.001 \)). There were no significant differences in donor gender, donor type, KT number, dialysis type before KT, cause of end-stage renal disease, and immunosuppressant for maintenance.

Clinical outcome comparisons between the BKVAN group and control group

The proportion of biopsy-proven acute rejection was significantly higher in the BKVAN group compared with the control group (66.7% vs. 12.1%, \( P < 0.001 \)). There was no significant difference in follow-up duration between the two groups. In Kaplan-Meier analysis, death-censored graft survival rate in the BKVAN group was significantly lower than that in the control group (\( P < 0.001 \)) (Fig. 2A). For cases of graft failure, the proportion of acute and chronic rejection was significantly higher in the BKVAN group compared with the control group, but the proportions of functioning allograft, recurrent glomerulonephritis, and infection were significantly lower in the BKVAN group compared with the control group (Table 2).

Patient survival rate in the BKVAN group was also significantly lower than that in the control group (\( P < 0.001 \)) (Fig. 2B). Patient death analysis indicated that the proportions of cardiovascular disease and infection were higher in the BKVAN group, and those of cerebrovascular

**Table 2. Comparison of clinical outcomes between the BKVAN and control groups in kidney transplantation**

| Variable                        | BKVAN group (n = 15) | Control group (n = 356) | \( P \) value |
|---------------------------------|----------------------|-------------------------|--------------|
| Biopsy-proven acute rejection   | 10 (66.7)            | 43 (12.1)               | < 0.001      |
| Cause of graft failure          | 10                   | 43                      | 0.095        |
| Acute rejection                 | 3 (30.0)             | 5 (11.6)                |              |
| Chronic rejection               | 5 (50.0)             | 7 (16.3)                |              |
| Functioning allograft           | 2 (20.0)             | 23 (53.5)               |              |
| Recurrent glomerulonephritis    | 0                    | 6 (14.0)                |              |
| Infection                       | 0                    | 2 (4.7)                 |              |
| Cause of patient death          | 5                    | 26                      | 0.487        |
| Cardiovascular disease          | 1 (20.0)             | 4 (15.4)                |              |
| Infection                       | 2 (40.0)             | 15 (57.7)               |              |
| Cerebrovascular accident        | 2 (40.0)             | 2 (7.7)                 |              |
| Hepatic failure                 | 0                    | 1 (3.8)                 |              |
| Malignancy                      | 0                    | 1 (3.8)                 |              |
| Others                          | 0                    | 3 (11.5)                |              |
| Follow-up duration (mo)         | 88.8 ± 57.9          | 99.0 ± 51.5             | 0.451        |

Values are expressed as number (%), number only, or mean ± standard deviation.

BKVAN, BK virus-associated nephropathy.

**Figure 2.** Comparison of (A) death-censored graft survival rate, and (B) patient survival rate between kidney transplant recipients with BK virus associated nephropathy and those without BK virus-associated nephropathy (BKVAN).
accident, hepatic failure, and malignancy were lower in the BKVAN group compared with the control group, but there were no significant differences between the two groups (Table 2).

In the Cox proportional hazard model, BKVAN and rejection were independent risk factors for graft failure after adjusting for recipient age, HLA mismatch number, antithymocyte induction, and deceased donor KT (Table 3).

In the subgroup analysis, the death-censored graft survival rate of KTRs with BKVAN accompanying acute rejection was significantly worse in comparison with KTRs with BKVAN without acute rejection and without BKVAN, regardless of acute rejection ($P < 0.001$) (Fig. 3).

**Discussion**

The rate of allograft loss in our center was higher than the above-mentioned average (66.7% vs. 46.2%). Short-term graft survival was good, but long-term graft survival was poor during the follow-up period (100% at 1 year, 92.9% at 3 years, 84.4% at 5 years, and 36.9% at 10 years). In particular, BKVAN patients with acute rejection had worse prognoses. BKVAN patients with acute rejection might progress to late graft failure without recovery of allograft dysfunction due to uncontrolled rejection. The prognosis of BKVAN was poor when the pathologic stage was more progressive [8], serum creatinine level was increased at diagnosis, or acute rejection occurred [11]. Because pathologic stage was directly associated with prognosis of BKVAN, early detection of BKVAN is very important before changes in pathologic findings [8].

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**Table 3. Risk factors associated with graft failure in kidney transplantation (KT)**

| Variable                        | Univariate |     |     |     |
|--------------------------------|------------|-----|-----|-----|
|                                 |            | HR  | 95% CI   | $P$ value |
| Recipient age at KT             |            | 0.991 | 0.959–3.294 | 0.588 |
| Recipient male gender           |            | 1.519 | 0.791–3.294 | 0.289 |
| HLA mismatch number             |            | 1.297 | 0.996–1.689 | 0.054 |
| ATG induction                   |            | 7.294 | 1.355–39.273 | 0.021 |
| BKVAN                           |            | 10.901 | 4.777–24.875 | < 0.001 |
| Rejection                       |            | 4.976 | 2.363–10.478 | < 0.001 |
| Deceased donor kidney transplantation |        | 2.364 | 1.107–5.052 | 0.026 |

ATG, antithymocyte globulin; BKVAN, BK virus-associated nephropathy; CI, confidence interval; HLA, human leukocyte antigen; HR, hazard ratio.

**Table 4. Comparison of pathologic findings between at diagnosis and at follow-up for BK virus associated nephropathy with acute rejection by allograft kidney biopsies**

| Variable | Biopsy at diagnosis of BKVAN | Follow-up biopsy | $P$ value |
|----------|-----------------------------|------------------|-----------|
| i score  | 1.2 ± 0.8                   | 1.7 ± 0.9        | 0.096     |
| ci score | 0.7 ± 0.7                   | 0.8 ± 0.6        | 0.678     |
| t score  | 1.2 ± 0.6                   | 1.7 ± 0.8        | 0.052     |
| ct score | 0.6 ± 0.7                   | 0.6 ± 0.8        | 1.000     |

Values are expressed as mean ± standard deviation.

BKVAN, BK virus-associated nephropathy.
ever, in our study, there were no significant differences according to pathologic stage or allograft function when BKVAN was diagnosed, unlike the results of other studies. It is possible that this difference could be attributable to the small number of BKVAN patients.

In most cases of BKVAN with acute rejection, acute rejection was first treated by steroid pulse therapy. Because the pathologic findings of the patients with acute rejection showed acute cellular rejection, we also performed steroid pulse therapy, similar to other studies [5]. MMF was discontinued in most patients after steroid pulse therapy. Tacrolimus was either reduced or replaced with cyclosporine. Sirolimus was added when the dosage of tacrolimus was reduced [3]. Two out of the 3 patients taking sirolimus maintained a stable renal function. In our study, 9 of the 10 BKVAN cases were accompanied by acute rejection, and all progressed to graft failure. One patient without acute rejection consistently used MMF without dosage reduction. BKVAN with acute rejection was associated with poor prognosis. One of the 5 patients with BKVAN in the non-graft failure group had acute rejection; tacrolimus was replaced with cyclosporine after steroid pulse therapy, and allograft function was stable.

In our study, the proportion of male recipients was significantly higher in the BKVAN group compared with the control group, and recipient age in the BKVAN group tended to be older than in the control group. The results were consistent with other studies [9]. The number of HLA mismatches tended to be higher in the BKVAN group compared with the control group, and the proportion of antithymocyte globulin as an induction immunosuppressant was significantly higher in the BKVAN group compared with the control group. These results were also consistent with other studies [9]. It is known that deceased donors, recipients over 50 years of age, and recipients with diabetes mellitus are risk factors for BKVAN; in our study, there were no significant differences between BKVAN and control groups [9]. However, incidence of acute rejection was significantly higher in the BKVAN group compared with the control group.

During a 10-year follow-up period, the prognosis of BKVAN was poor compared to the control group. The 10-year graft survival rate in the BKVAN group was 36.9%, and that in the control group was 93.1%. The 10-year patient survival rate in the BKVAN group was 66.9%, and that in the control group was 92.9%. Rejection was the most common cause of graft failure and infection was the most common cause of patient death in both groups.

In the subgroup analysis, we divided the KTRs who underwent allograft kidney biopsies into four groups as follows: Those with or without BKVAN and with or without acute rejection. Fig. 3 shows that death-censored graft survival rate in the group without BKVAN and with acute rejection was lower in comparison with the group with BKVAN and without acute rejection. In general, the risk of graft failure in BKVAN is high; however, in our study, there were few cases of graft failure in the group with BKVAN and without acute rejection, and the incidence of graft failure due to uncontrolled acute rejection in the group without BKVAN and with acute rejection was high. This result could be attributable to the small size of the BKVAN group. Death-censored graft survival rate of KTRs with BKVAN accompanying acute rejection was significantly worse in comparison with KTRs with BKVAN without acute rejection and without BKVAN, regardless of acute rejection. In the case of BKVAN with acute rejection, follow-up biopsy was performed to show the therapeutic effect, since the patients were not able to undergo more aggressive anti-rejection treatment, except steroid pulse therapy. Most pathologic findings showed that acute rejection deteriorated after treatment. Therefore, the reasons for the poor prognoses might be graft failure due to inadequate anti-rejection treatment for acute rejection that accompanied BKVAN.

There were some limitations to this study. First, our study has a retrospective design, and there is a high possibility of selection bias. Second, the sample size of our study was too small to clearly demonstrate any impact of BKVAN on clinical outcome; therefore, additional large-scale studies are needed. Third, we did not perform allograft kidney biopsies in 211 of the 258 patients with positive urine decoy cells, because we performed biopsies in cases of allograft dysfunction or proteinuria in positive urine decoy cells. Therefore, we excluded the 211 patients in our study because we could not predict the presence of BKVAN. It is possible that there would have been more patients who had BKVAN because allograft function could be improved by reduction or change of immunosuppressants without allograft biopsies. Therefore, well-designed screening protocols to diagnose BKVAN are needed. Fourth, we did not perform allograft kidney biopsies for all patients who were treated with
BKVAN and only performed them in cases of BKVAN with acute rejection. When allograft dysfunction was consistent, we could not determine whether the BKVAN was uncontrolled or the treatment for acute rejection was not completed. Finally, surveillance tests, such as plasma or urine BKV DNA PCR, were not regularly performed in all patients with BKVAN.

In conclusion, the long-term prognosis of BKVAN with acute rejection was very poor because of graft failure caused by inadequate treatment for acute rejection, considering BKVAN. Therefore, we should carefully monitor the allograft status of KTRs through regular surveillance tests after treatment with BKVAN with acute rejection.

Conflicts of interest

All authors have no conflicts of interest to declare.

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