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Original Article

Tacrolimus Intrapatient Variability in BK Virus Nephropathy and Chronic Calcineurin Toxicity in Kidney Transplantation

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ABSTRACT. Intrapatient variability (IPV) in tacrolimus has been increasingly acknowledged as a risk factor for poor graft survival after kidney transplantation. Although past studies have mainly accounted for IPV in acute or chronic rejection states as due to underimmunosuppression, this is not yet clear. So far, tacrolimus IPV for BK virus-associated nephropathy (BKVN) and chronic calcineurin inhibitor toxicity (CNIT) has not been investigated. Here, we evaluated IPV in tacrolimus for BKVN and chronic CNIT, which are mainly considered as overimmuno-suppression states. In this case–control study, kidney allograft biopsies conducted between 1998 and 2018 were included, with patients grouped by biopsy results as BKVN alone group, CNIT alone group, and normal graft function (control group). IPV was estimated as mean absolute deviation. Our study groups included 25 kidney transplant recipients with BKVN alone, 91 patients with CNIT alone, and 60 patients with normal 5-year graft survival (control group). In analyses of IPV in tacrolimus six months before graft biopsy, IPV was highest in the BKVN group (P = 0.001).The BKVN group also had the highest IPV in tacrolimus at 12 months after biopsy (P = 0.001), with all pairwise comparisons statistically different between groups. At 12 months after biopsy, five patients (20%) in the BKVN group and 10 patients (10.9%) in the CNIT group had graft loss. Among other risk factors, BKVN and chronic CNIT are consequences related to high IPV. Quantification of IVP for tacrolimus in clinical practice would help to optimize kidney transplant outcomes.

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Introduction

Since its discovery, tacrolimus has been widely used in the world and in Turkey in kidney transplantation as a part of the immuno-suppressive regimen.[1,2] However, therapeutic monitoring of tacrolimus remains complicated because a narrow margin exists between

adequate immunosuppression and toxicity. Daily blood trough concentrations are currently used for dose adjustments in clinical practice. Because of its pharmacokinetics, high inter-patient variability and intrapatient variability (IPV) can limit the use of blood drug concentration to set the drug dosage. IPV is defined as the fluctuation in tacrolimus concentrations within an individual patient over a certain period during which the tacrolimus dose is unchanged.[2] High IPV is related to many factors, extending from diet to ethnic differences, and is associated with poor long-term outcomes.[2-4] Fluctuations in concentrations can result inpatients being at risk of underexposure and rejection or tacrolimus toxicity in cases of overexposure.

BK virus-associated nephropathy (BKVN) may be a good model to represent excessive immunosuppression, which predisposes eventually to graft loss.[5] Chronic calcineurin inhibitor toxicity (CNIT) is the other side of overexposure, resulting in irreversible damage to the renal architecture.[6]We hypothesized that fluctuations in tacrolimus blood trough levels may be associated with the coexistence of BKVN or CNIT. In this study, we aimed to investigate tacrolimus IPV in BKVN and CNIT as a surrogate marker for estimating over immunosuppression in the kidney transplant setting.

Methods

Study population and laboratory data

Our retrospective cohort study included kidney transplant recipients who received transplants between 1998 and 2018 at the Baskent University Hospitals (Ankara, Turkey). We reviewed 1598 graft biopsy reports among our patients. Of total reports, there were 89 BKVN-related reports and 614 CNIT-related reports. The remaining 895 biopsy reports included acute humoral rejection, acute cellular rejection, transplant glomerulopathy, and others.

Patients were included in the analysis if (1) they were 18 years old or older at the time of the transplant, (2) they received a living kidney donation, (3) they had been maintained on tacrolimus + mycophenolate mofetil + steroid as immunosuppression at a stable dose for at least 12 months before the biopsy,(4) they had no rejection episodes during the preceding 12 months before graft biopsy, and (5) graft biopsies had been performed at least 12 months after transplant and did not include any rejection or other pathological findings concomitantly. Patients with missing data and who had <5 times of blood tacrolimus level measurements during the preceding six months and one year after graft biopsy were also excluded.

In total, 25 patients with BKVN alone and 91 patients with CNIT alone were included in our study. A flowchart diagram of patient election is presented in Figure 1. As a control group, we analyzed 60 patients who had 5-year graft survival and never had any rejection episodes. None of the patients in the control group needed a graft biopsy. We gathered demographic information and laboratory data from health records and analyzed laboratory data included serum creatinine, serum albumin, and C-reactive protein levels immediately before the biopsy. For patient outcomes, we assessed graft loss (defined as starting hemodialysis or peritoneal dialysis) over the 12 months.

Tacrolimus blood levels and intrapatient variability

Mean tacrolimus blood concentration and mean daily drug dosage during the six months immediately before the graft biopsy were analyzed. To calculate the IPV for each patient, at least five tacrolimus blood samples had to be available in the preceding six months and the following 12 months after the graft biopsy. Tacrolimus IPV was quantified as the mean absolute deviation (MAD%). The MAD% was calculated as follows[3]:

$$MAD\% = 1/n \sum_{(i=1)}^{n} (|Xi - m(x)|)$$

X100, where m(X) represents average value of the data set, *n* represents the number of data values, and Xi represents data values in the set.

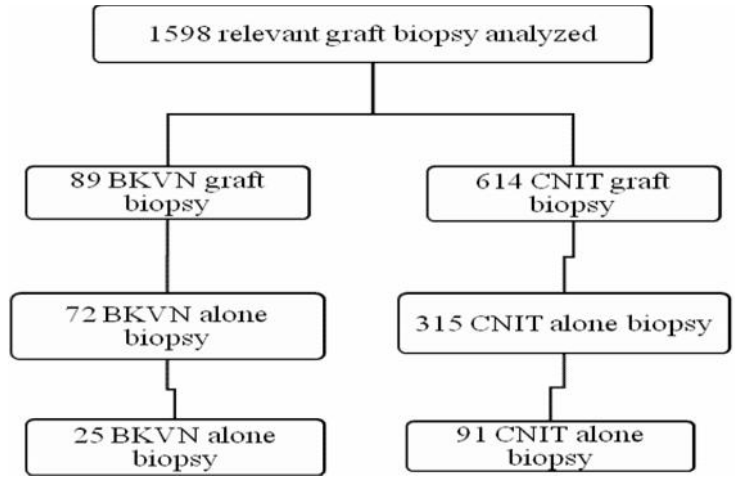

Figure 1. Flow-chart diagram of study design.

Compliance with ethical standards

This study was approved by Baskent University Institutional Review Board (Project No:KA21/66). Data had been obtained as a part of standard patient care and were analyzed in an anonymized fashion. Informed consent was taken from all patients when they applied to our hospital stating retrospective use of their data.

Statistical Analyses

The distribution of baseline demographics was summarized using descriptive statistics. Continuous variables are expressed as median and interquartile range. To evaluate the differences between median values, we used Kruskal–Wallis test for three groups, with pairwise comparisons for *posthoc* analysis. Chi-square tests were used for categorical variables. $P \leq 0.05$ was accepted as significant. Statistics were mainly calculated with the use of IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA).

Results

Twenty-five patients with biopsy-proven BKVN, 91 patients with biopsy-proven chronic CNIT, and 60 patients with normal graft function were analyzed. Among the total patients analyzed (n = 176), the mean age was 34.39 ± 11.77 years, and time to graft biopsy from transplant was 54.92 ± 30.58 months. Mean tacrolimus dose in the six-months before biopsy was 4.18 ± 2.20 mg/day, and mean blood trough level was 7.41 ± 3.89 µg/L. For analysis of mean IPV in tacrolimus during the six months before biopsy, rate was 30.7% ± 8.5%. In the 12 months after biopsy, IPV rate was 34.3% ± 13.1%. According to study groups, serum creatinine was higher in both the BKVN group (P = 0.001) and CNIT group (P = 0.001) than in the control group but similar between BKVN and CNIT groups (P = 0.821). C-reactive protein and albumin levels were similar between the three groups. Table 1 summarizes the demographic characteristics and laboratory data.

In the analysis of drug use, tacrolimus daily dosage in the six months before biopsy was similar between the BKVN group and the control group (P = 0.792).However, in the CNIT group, dosages were lower compared with both the BKVN group (P = 0.033) and control group (P = 0.012). Tacrolimus blood trough level during the past six months before biopsy was similar between the three groups (P = 0.219). For all three groups, IPV in tacrolimus for the preceding six months before biopsy was, in descending order, highest in the BKVN group (P = 0.001), followed by the

Table 1. Demographic and laboratory data of the study.

Parameters	BKV nephropathy (*n*=25)	CNI toxicity (*n*=91)	Normal graft function (*n*=60)	*P*
Age, year	43 (22)	30 (18)	35 (16)	0.011
No. of women (%)	14 (56%)	55 (60.4%)	35 (58.3)	0.913
Timing of renal biopsy after transplant, months	24 (23)	48 (44)		0.001
Laboratory data at time of biopsy				
Serum creatinine, mg/dL	2.12 (1.54)	2.47 (1.57)	1.02 (0.30)	0.001
Serum albumin, g/dL	4 (0.8)	3.89 (0.9)	4.2 (0.38)	0.056
Serum CRP, mg/L	7.2 (20.33)	3.2 (12.30)	5 (6.8)	0.233
Mean tacrolimus blood trough concentration (last 6 months), µg/L	7.6 (3.6)	6.5 (3.9)	7.28 (1.99)	0.219
Mean tacrolimus dose(last 6 months), mg/day	4 (5.5)	3 (3)	4 (1.0)	0.014
IPV last 6 month, No. (%)	39 (11)	30.51 (9.37)	30.1 (9.97)	0.001*
IPV following 1 year, No. (%)	54.9 (26.64)	33.4 (10.82)	28.6 (25.89)	0.001*
1st year graft loss, No. (%)	5 (20%)	10 (10.9%)	0 (%)	

BKV: BK virus, CNI: Calcineurin inhibitor, CRP: C-reactive protein, IPV: Intrapatient variability.
All data are shown as median and interquartile range, unless otherwise indicated. *Groups were statistically different from each other in pairwise comparisons.

CNIT group (P = 0.001) and then the control group (P = 0.001). IPV in tacrolimus for the 12 months after biopsy was also highest in the BKVN group (P = 0.001), with all pair-wise comparisons statistically different between groups. For the 12 months after biopsy, five patients (20%) in the BKVN group and 10 patients (10.9%) in the CNIT group lost their graft. In patients who lost their graft, the IPV in tacrolimus was higher in the BKVN group than in the CNIT group (P = 0.013). IPV in tacrolimus results according to groups are shown in Figures 2 and 3.

Discussion

In this retrospective case–control study, we tested the hypothesis that high IPV in

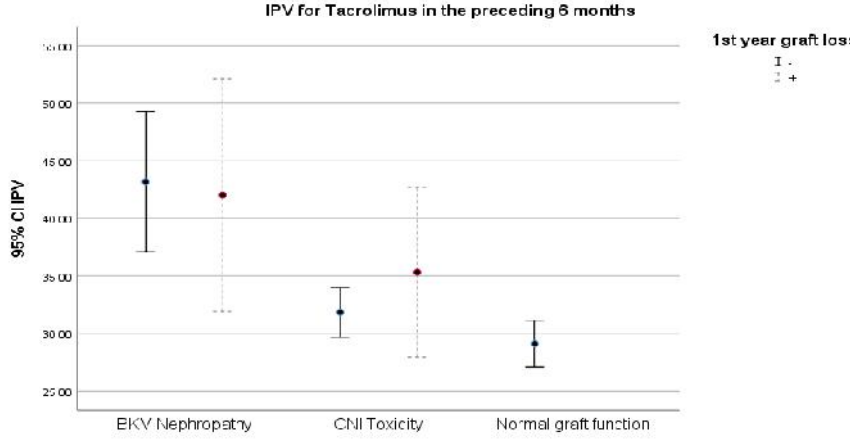

Figure 2. Intrapatient variability in tacrolimus during the preceding 6 months before biopsy according to patient groups.

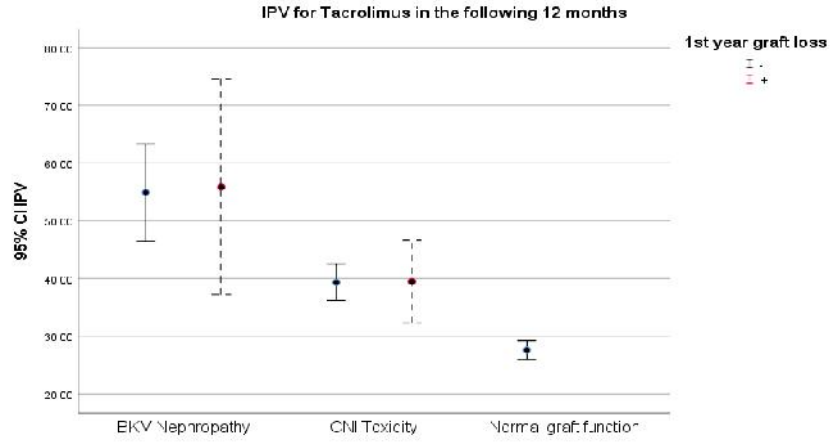

Figure 3. Intrapatient variability intacrolimus during the following 12 months after biopsy according to patient groups.

tacrolimus levels is associated with an overimmunosuppression state, as observed in BKVN or CNIT of the kidney allograft. We found that, irrespective of tacrolimus daily dose or blood trough levels, fluctuations in tacrolimus blood levels were prominent in BKVN in the pre-period of infection. In the follow-up period of BKVN and chronic CNIT, high IPV was also shown.

Fluctuations in tacrolimus are common in the 1st weeks or months after transplant due to numerous factors, with changes in maintenance drug doses.[7] Generally, after a couple of weeks or months, patients reach a more stable blood druglevel.[8] However, nonadherence, genetic factors, drug–drug interactions, diet, and generic formulations may lead to exceeding or falling below the therapeutic range of tacrolimus, resulting in high IPV.[9] Highly fluctuating tacrolimus trough concentrations, despite a stable dose, can also occur with no reason. In our study, we tried to minimize the occasions, resulting in high IPV. We excluded all patients with <12-month duration since transplant and patients with any rejection episode during the past 12 months, to prevent any incidences of drug–drug interactions. All of our patients were using twice daily formulations of tacrolimus.

Many studies have reported that higher IPV of tacrolimus is related to worse graft survival.[2-4,9-11] Some studies have reported a higher acute rejection rate in patients with a greater variation in intrapatient tacrolimus levels,[12,13] although some did not.[14,15] However, IPV in tacrolimus for those with BKVN and CNIT is still unknown. In their study, Shen et al analyzed BKVN and acute rejection together and reported that fluctuations in tacrolimus levels were noticeably associated with the coexistence of BKVN and acute rejection.[16] However, they compared BKVN alone with BKVN and acute rejection together and the immunosuppressant drug regimens used in their study were not standard as were ours. To our knowledge, our study is the first to analyze IPV in tacrolimus in patients with BKVN and CNIT separately and versus a control group to estimate whether high IPV is a predisposition to BKVN or chronic nephrotoxicity.

BKVN is a significant risk factor for premature allograft loss, with an incidence of up to 10%–15% after kidney transplant.[17] Beyond the many risk factors, the intensity of immunosuppression is the dominant risk for viral replication and nephropathy.[18] However, no specific immunosuppressive drug or regimen has been definitively associated with clinically significant infection.[19-21] In addition to the known risk factors related to BKVN,[22] the more obscure reasons for overimmunosup-

pression are still unclear. The pharmacokinetic changes of drugs rather than the blood levels might be responsible for uncontrolled immunosuppression without apparent toxic side effects.

In our study, living donor kidney transplant recipients with high IPV ended with BKVN after one year of graft function without complications. In addition, their tacrolimus daily dose and blood trough levels were similar to those shown in patients with normal graft function. Recent studies have shown that graft loss after BK virus infection is mainly related to increased risk of acute rejection due to decreased immunosuppression.[23] However, in the period before infection, it is enigmatic to see acute rejection concomitantly. In their study, Seifert et al[22] found that polyomavirus reactivation was associated with early *de novo* antibodies to kidney-specific self-antigens fibronectin and collagen IV. Although the exact pathway is unclear, the same mechanism increases the risk of acute rejection and infection.[24] The findings of Seifert et al were in agreement with our findings that high IPV during the BKVN course might be related to this unstable immune response. However, further studies are warranted to elucidate this argument.

In the other aspect of oversuppression, chronic nephrotoxicity cannot be disregarded because it can result in irreversible chronic progressive renal disease. However, the evidence of CNIT being associated with higher systemic drug dosages is not strong.[25] In fact, it was reported that low tacrolimus levels are highly associated with chronic tubulointerstitial damage.[6] Investigations have revealed that progression of chronic damage is more the result of local renal factors than of overexposure to drugs, with inter- and intra-variabilities of calcineurin inhibitors strengthening these local renal factors.[24] In the current literature, there is no study estimating IPV in patients with CNIT. We found that IPV was lower than in the BKVN group ($P = 0.001$) but higher than in the control group ($P = 0.001$) during the preceding six months. However, IPV was still high after 12 months, especially in patients who lost their graft due to CNIT. Prevention and eventually therapy for calcineurin inhibitor nephrotoxicity are aimed at lowering total systemic blood levels and decreasing local renal exposure to the calcineurin inhibitors or their metabolites.[26,27] However, it is reasonable to speculate that high IPV might affect these local renal factors and should be corrected during routine posttransplant follow-up of kidney transplant recipients.

There are some limitations to the present study. Sample size was small in the BKVN group due to rareness of the disease. Second, due to our retrospective study design, we could not analyze patients for other reasons of kidney rejection during the 12-month follow-up. Third, the diagnoses were all based on kidney pathology instead of urine or blood examinations. However, this is the first study to identify that high drug level variability might be related to both BKVN and CNIT.

In conclusion, our results demonstrated that fluctuations in tacrolimus levels were relevant in BKVN and CNIT. Further studies are needed to better understand the interactions between infection, sensitization, and toxicity. Beyond routine therapeutic drug monitoring, IPV in tacrolimus is easy to determine, allowing testing to be routinely incorporated into everyday clinical practice as a tool for optimizing transplant outcomes.

Conflict of interest: None declared.

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