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1. Title: Incidence and Risk Factors for High-level BK viruria -A Single Center Study in China

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Abstract

Background BK Virus Allograft Nephropathy (BKVAN) is a serious complication after kidney transplantation, and the effect of pre-emptive intervention for high-level BK viruria has been verified, but the protocols after kidney transplantation for early identification of high-level viruria is lacking.

Methods This was a single-center respectively study. The clinical data of the kidney transplant recipients and their donors in our center from January 1, 2015 to December 31, 2018 were collected. According to the qPCR results of BK virus DNA loads in urine samples, the patients were divided into high-level BK Viruria Group (Group A) and none high-level BK Viruria Group (Group B). Significant variables were screened out by univariate analysis, and then the results were incorporated into multivariate logistic regression model to analyze the independent risk factors of high-level BK viruria.

Results A total of 262 recipients were included in the study. The incidence of high-level BK viruria was 13.4% (n=35), and the median time of detection was 181(range 91~1119) days. Univariate analysis showed that the donor type (χ^2=21.770, P < 0.001), history of ATG/ATG-F application (χ^2=4.543, P=0.033), Acute Rejection (AR) (χ^2=8.313, P=0.004) and Delayed Graft Function (DGF) (χ^2=21.170, P < 0.001) were related with high-level BK viruria. After the inclusion of multivariate logistic regression model, the results showed that brain and cardiac deceased donors (P=0.032,OR=3.927, 95%CI:1.122~13.746), AR (P=0.022,OR=4.709, 95%CI:1.253~17.697) and DGF (P=0.001,OR=6.682, 95%CI:2.288~19.518).

Conclusions Donation of Brain and Cardiac Deceased, history of AR, DGF were independent risk factors for high-level BK viruria after kidney transplantation.

Key Words Kidney Transplantation, BK Viruria, Risk Factors, Incidence

Introduction

BKVAN is a severe disease caused by BK virus (BKV) infection or reactivation, which often impairs kidney function irreversibly, and is more common in kidney transplant recipients[1]. The usual progression of infection begins with BK Viruria and progresses to BK Viremia eventually leads to BKVAN. The importance of prevention is underscored by the lack of a specific treatment regimen for BKVAN. In 2013, American Society of Transplantation[1] recommended starting intervention at the stage of high-level BK Viremia, but in a few studies, pre-emptive treatment with BK Viruria seemed to be more advantageous[2-4]. Early identification of high-risk patients plays an important role in prevention. Currently, reported risk factors are usually reported by a single center, and mainly focus on the analysis of BK Viremia, with large differences in results, which always makes people confused.

This study summarized the incidence of high-level BK viruria from 2015 to 2018, and analyzed its risk factors, so that it could identify patients at an early stage and give treatment to prevent the occurrence of BKVAN.

Patients and Methods

Patients' Groups

In this study, we retrospectively collected the data of kidney transplant recipients from January 1, 2015 to December 31, 2018 at Jiangxi Provincial People's Hospital Affiliated to Nanchang University. This study was approved by the Ethics Committee of Jiangxi Provincial People's Hospital (Serial no. 2015094).According to the different

monitoring results of BKV DNA loads in urine after transplant, the patients were divided into two groups: high-level BK viruria (Group A)(BKV DNA loads≥E+07copies/ml),none high-level BK viruria (Group B)(BKV DNA loads＜E+07copies/ml or none BKV DNA in urine samples)。The exclusion criteria were：1. Diagnosed with BK viremia;2.No regular follow-up data were kept after the operation;3.The patient died or lost the transplanted kidney during the study period.

Monitor Protocols

We chose a more rigorous post-operative monitoring regimen than the guidelines. Regular urine BKV monitoring was carried out for kidney transplant recipients after transplant, and the monitoring frequency was once a month for 1^{st}-9^{th}month after transplant, once every 3 months for 9^{th} month-2^{nd} year, and once a year for 2^{nd}-5^{th}year. The urine BKV DNA loads was detected by quantitative Polymerase Chain Reaction (qPCR), and the plasma BKV DNA loads was detected when the urine BKV DNA loads≥ E+07 copies/ml. qPCR detection instrument: AGSAFD-9600, China Public Health (Shanghai) Biotechnology Co., LTD., Shanghai, China (detection threshold > 2000 copies/mL); Reagent Kits: BK virus nucleic acid detection kits (PCR fluorescent probe method), SINOMD Gene Detection Technology Co., LTD., Beijing, China.

Data Collected

The clinical data of all the recipients and their donors were collected. Includes: donor factors (sex, age, BMI, renal type, the serum creatinine before organ obtain, left or right kidney), recipients factors (sex, age, BMI, preoperative dialysis method,

ATG/ATG-F medical history, history of AR, DGF, the infection in 30 days after operative, immunosuppression maintain protocols, number of transplant, BK virus DNA loads within urine, the time of high-level BK viruria), immune factors (HLA mismatching points, cold ischemia time, warm ischemia time).

Statistical Analysis

The statistical analyses were performed with IBM SPSS software, version 25.0(Armonk, NY, United States). The results of continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR) and numerical (percentages) for categorical variables. The chi-square test and the independent sample T-test were used for the single-factor comparison between the two groups. The results of univariate analysis ($P < 0.1$) were included in the multivariate Logistic regression model analysis, and the forward LR method was used to screen the variables and eliminate the unintentional variables. $P < 0.05$ was considered to indicate statistically significant.

Results

Demographics and Clinical Characteristics of Recipients and Donors

A total of 262 patients were included in this study, including 35 in group A and 227 in group B. All the recipients were negative for panel reactive antibody and complement dependent cytotoxicity before transplant. The demographics and clinical characteristics of all subjects are shown in Table 1. The prevalence of high-level BK viruria was 13.4% (n=35), and the median time of detection was 181(range 91~1119)

109 days. Figure 1 shows the distribution of the onset time of 35 patients with high-level
110 BK viruria. The median follow-up time for all recipients was 1004 (range 372~1954)
111 days.

112 Table 1. Demographics and Clinical Characteristics of Recipients and Donors

Variates		All recipients n=262	Group A n=35	Group B n=227	χ^2 or t value	P value
Donors						
Sex (n, %)	Male	197,75.2%	28,80%	169,74.4%	0.501	0.479
	Female	65,24.8%	7,20%	58,25.6%		
Age (n, %)	＜18	27,10.3%	3,8.6%	24,10.6%	0.618	0.734
	18~59	232,88.5%	32,91.4%	200,88.1%		
	≥60	3,1.1%	-	3,1.3%		
BMI (n, %)	＜18kg/m^2	27,10.3%	3,8.6%	24,10.6%	0.873	0.832
	18-23.9 kg/m^2	161,61.5%	24,68.6%	137,60.4%		
	24-27.9 kg/m^2	64,24.4%	7,20%	57,25.1%		
	≥28kg/m^2	10,3.8%	1,2.9%	9,4.0%		

							<
	Donor type (n, %)	Live	40,15.3%	4,11.4%	36,15.9%	21.7	0.00
							1
		Brain Death	12,4.6%	1,2.9%	11,4.8%		
		Cardiac Death	138,52.7%	9,25.7%	129,56.8%		
		Brian-Cardiac Death	72,27.5%	21,60.0%	51,22.5%		
	Serum Creatinine(Mean±SD)		93.2±54.2μmol/L	94.5±65.2μmol/L	93.0±52.5μmol/L	- 0.152	0.88 0
	Left or right kidney	Left	141,53.8%	19,54.3%	122,53.7%	0.004	0.95 2
		Right	121,46.2%	16,45.7%	105,46.3%		
Recipients	Sex (n, %)	Male	189,72.1%	22,62.9%	167,73.6%	1.731	0.18 8
		Female	73,27.9%	13,37.1%	60,26.4%		
	Age (n, %)	＜18	2,0.8%	-	2,0.9%	0.365	0.83 3
		18~59	254,96.9%	34,97.1%	220,96.9%		
		≥60	6,2.3%	1,2.9%	5,2.2%		
	BMI (n, %)	＜18kg/m^2	36,13.7%	3,8.6%	33,14.5%	3.026	0.38 8

	18-23.9 kg/m^2	187,71.4%	28,80%	159,70.0%		
	24-27.9 kg/m^2	36,13.7%	3,8.6%	33, 14.5%		
	≥28 kg/m^2	3,1.1%	1,2.9%	2, 0.9%		
Dialysis (n, %)	Hemodialysis	209,79.8%	25,71.4%	184,81.0%	2.382	0.304
	Peritoneal	51,19.5%	10,28.6%	41,18.1%		
	others	2,0.8%	-	2,0.9%		
ATG/ATG-F (n, %)	No	176,67.2%	18,51.4%	158,69.6%	4.543	0.033
	Yes	86,32.8%	17,48.6%	69,30.4%		
AR (n, %)	No	217,82.8%	23,65.7%	194,85.5%	8.313	0.004
	Yes	45,17.2%	12,34.3%	33,14.5%		
DGF (n, %)	No	215,82.1%	19,54.3%	196,86.3%	21.170	< 0.001
	Yes	47,17.9%	16,45.7%	31,13.7%		
Infection within 30 days after surgery (n, %)	pulmonary infection	14,5.3%	3,8.6%	11,4.9%	3.936	0.140

		urinary tract infection	14,5.3%	4,11.4%	10,4.4%	
		others	233,88.9%	28,80%	205,90.7%	
Immune factors	Immunosuppressive maintenance regimen (n, %)	TAC+MMF+Pred*	259,98.9%	34,97.1%	225,99.1%	1.046	0.306
		Others	3,1.1%	1,2.9%	2,0.9%		
	the number of kidney transplant (n, %)	First	258,98.5%	35,100%	222,98.2%	0.626	0.731
		Second	4,1.5%	-	4,1.8%		
	HLA mismatching(Mean±SD)		2.0±1.1	1.9±1.2	2.0±1.0	0.745	0.457
	cold ischemia time (Mean±SD)		10.2±4.7h	9.8±4.2h	10.3±4.8h	0.555	0.576
	Warm ischemia time (Mean±SD)		4.5±1.3min	4.8±1.2min	4.5±1.3min	-1.466	0.149

113 *:Tacrolimus+ Mycophenolate Mofetil+ Prednison(Tac+MMF+Pred)

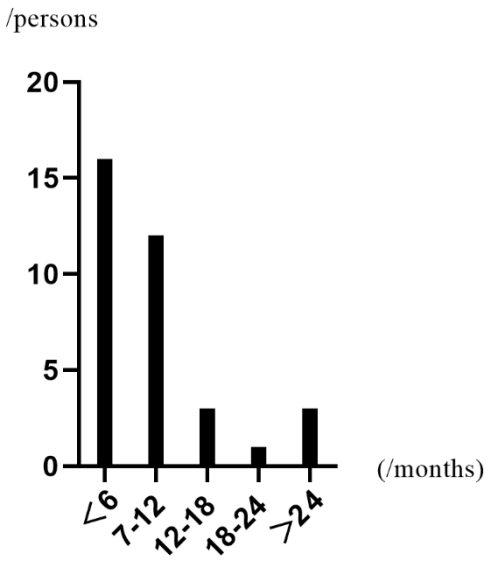

Figure 1. onset time distribution of high-level BK viruria

This graph shows the highest incidence within 6 months of the transplant, decreases over time, and then increases again 2 years later.

Results of univariate and multivariate analysis

In Table 1, we indicated the univariate analysis results of different variables, and the P-value < 0.1 variable was input into the logistic regression model for multi-factor analysis to identify the independent risk factors. The treatment history of ATG/ATG-F, AR, DGF and donor type were included in the regression model. The results showed that the treatment history of ATG/ATG-F (OR: 0.339; 95% CI: 0.084~1.370; P = 0.129), the AR (OR: 4.709; 95% CI: 1.253~17.697; P = 0.022), DGF (OR: 6.682;95% CI: 2.288~19.518;P=0.001) and Donation of Brain and Cardiac deceased (DBCD) (OR: 3.927;95% CI: 1.122~13.746;P=0.032) (Figure 2).

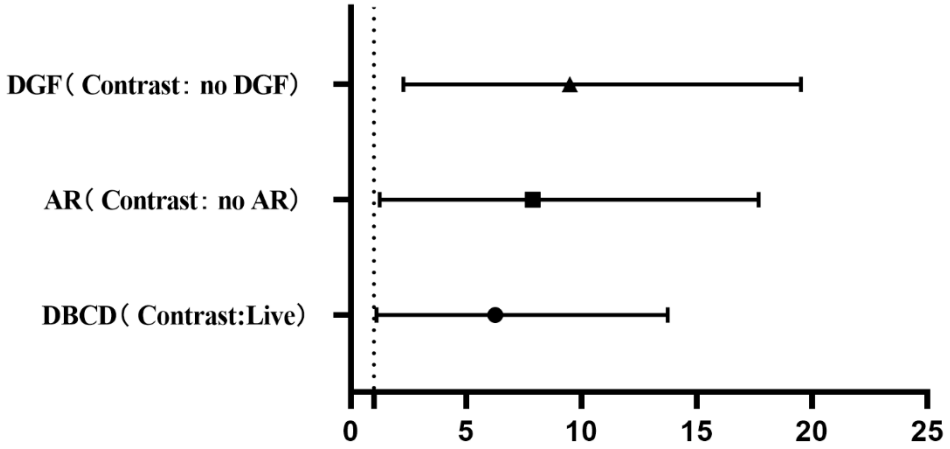

Figure 2. OR values for independent risk factors for high-levels of BK viruria

DGF: Delayed Graft Function), OR=6.682; 95%CI, 2.288~19.518; P=0.001;AR: Acute Rejection, OR=4.709; 95%CI, 1.253~17.697; P=0.022;DBCD: Donation of Brain and Cardiac Deceased, OR=3.927; 95%CI, 1.122~13.746; P=0.032.

Discussion

By monitoring the urine BKV DNA loads regularly after kidney transplant, we found that 13.4% of the patients (n=35) were diagnosed with high-level BK viruria at the median time of 181 days in our center. In 2020, some scholars summarized that the incidence of BK virus infection in Asia, they found that the incidence of BK viruria after kidney transplantation was between 5.9%~86.9%[5]. Although our data is also within this range, the wide range also affects the accuracy of this data. In fact, we have to admit that the existing data are all reported by single center, there are too many uncontrollable factors leading to huge differences in the results. Earlier, some scholars also reported the prevalence of BKV infection among healthy people. Atonsson et al.[6] reported that the serum positive rate of BK virus in Australians was as high as 99% in

people between 25 and 60 years old. Gossai et al.[7] investigated the prevalence of polyomavirus in the United States and found that the serum positive rate of BK virus was 87.6%. These reports reveal differences in the prevalence of BK virus infection in time and space. To be sure, the highest incidence of BK viruria in renal transplant recipients was within 6 months of surgery, in accordance with the other centers have reported[8].

It is significant to identify high-level BK viruria patients. We began to carried out the pre-emptive intervention in 2015 to intervened the high-level BK viruria to prevent the occurrence of BKVAN, and the results were satisfactory. Among the 38 patients, BK viruria was effectively controlled in 32 patients (84.2%) within 1 year of treatment, and the remaining 6 patients (15.8%) also showed no infection progression, and no rejection reaction occurred in all patients after the immunosuppression intensity was reduced [The article is under submission]. Some researchers have also found that BK viruria also causes serum creatinine elevation by analyzing the survival of renal transplant recipients infected with BK virus[9]. So, we conducted this study to explore the risk factors for high-level BK viruria.

As we know, no relevant studies have been reported internationally in this filed, so we selected some variables that may influence the occurrence of high-level BK viruria for analysis. The final results showed that DBCD, AR and DGF were independent risk factors for high-level BK viruria. AR and DGF were the expected results, and AR and DGF were also independent risk factors for BK viremia after renal

transplantation[10]. But DBCD surprised us. As was known to all, the donors' source in China has undergone a great change in the 21st century. Influenced by traditional culture and religion, the development of DBD donors has been greatly hindered, which also severely limits the quantity and quality of our transplant work. Therefore, the criteria of donation in Chinese was developed, in order to solve the problem of the extreme shortage of donors in China. DBCD is the third type of donor in China (C-III), which is similar to category 4 in Maastricht criteria[11]. Theoretically, we think that DCD might be one of the risk factors for the progression of BK virus infection. The incidence of DGF and primary nonfunction was significantly increased because DCD donors experienced hemodynamic disorders and the attack of underlying diseases. Generally, DBCD is similar to DBD, and the quality of kidney is significantly higher than that of DCD. So maybe DCD is more closely associated with infection[10, 12]. This makes us confused, whether it is the bias caused by the small number of cases or the specificity of DBCD, which needs to be confirmed by more studies.

This study reports the risk factors for high-level of BK viruria after renal transplantation, filling in the gaps in this field preliminarily, but there are also some deficiencies inevitably. The single center retrospective study, the insufficient sample size was still the limitation of its quality. Due to the limitation of objective factors, we couldn't compare all relevant factors. For example, there are limited types of immunosuppressive drugs that we use after transplant, induction therapy is not routine, donor-sourced BK virus surveillance has not been carried out, and so on.

Conclusions

In the first 6 months after kidney transplantation, enhanced monitoring frequency of BKV infection is necessary, and DBCD kidney, a history of AR/DGF are independent risk factors for high-level BK viruria, we can use that as a basis to identify patients early on and treat them. In future studies, prospective multicenter studies will always be an important direction in the field of BKV infection.

Abbreviations

BKVAN: BK virus allograft nephropathy, BKV:BK virus, qPCR: quantitative Polymerase Chain Reaction, AR: Acute Rejection, DGF: Delayed graft function, DBD: Donation of brain deceased, DCD: Donation of cardiac deceased, DBCD: Donation of brain and cardiac deceased, SD: Standard deviation, IQR: Interquartile range, Tac: Tacrolimus, MMF: Mycophenolate Mofetil, Pred: Prednison, OR: Odds Ratio, CI: Confidence interval.

Declarations

Ethics approval and consent to participate

This research has been approved by the ethics committee of Jiangxi Provincial People's Hospital Affiliated to Nanchang University (Serial no. 2015094). However, there were no informed consents were signed, due to the retrospective study, except for personal

information, only the clinical data of anonymous patients were collected without infringing the rights and interests of the patients.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflicts of interest.

Funding

Jiangxi Provincial Department of Science and Technology Key Research and Development Project Fund (grant no. 20151BBG70069).

Authors contributions

Rui Xiong, participated in research design, participated in the writing of the paper, participated in the performance of the research, participated in data analysis. final approval of the version to be published, agreement to be accountable for all aspects of

the work; Haimin Ye, participated in research design, participated in the revising of the paper, participated in data analysis. final approval of the version to be published, agreement to be accountable for all aspects of the work; Zhujing Liu, participated in research design, participated in the revising of the paper, participated in data analysis. final approval of the version to be published, agreement to be accountable for all aspects of the work; (5) Xinchang Li, participated in research design, participated in the revising of the paper, participated in the performance of the research, final approval of the version to be published, agreement to be accountable for all aspects of the work.

Acknowledgements

Not applicable.

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