

Original Article**Comparison of polyomavirus (BK virus and JC viruses) viruria in renal transplant recipients with and without kidney dysfunction**

*Shahram Taheri*¹, *Farshid Kafilzadeh*², *Maryam Shafa*³,
*Majid Yaran*⁴, *Mojgan Mortazavi*⁵, *Shiva Seirafian*¹,
*Shahzad Shahidi*¹, *Abdolamir Atapour*⁵

Abstract

BACKGROUND: Post-transplant infection with polyoma viruses (BK and JC viruses) is an important cause of graft loss and nephropathy. The objective of this study was to compare the frequency of BK and JC viruria in renal transplant recipients with and without graft dysfunction.

METHODS: In a case-control study, we selected 60 kidney transplant patients with and without graft dysfunction in the first two years after transplantation. Each group consisted of 30 patients evaluated for basic demographic and laboratory characteristics. First morning urine samples were sent for BK and JC virus detection with QIAamp DNA Mini Kit and real-time polymerase PCR. Chi-square test with Yates' correction, Student t-test and Mann-Whitney U test were used as indicated. P value of less than 0.05 was regarded as statistically significant.

RESULTS: Both groups were similar in age, gender, and time after transplant and pretransplant dialysis. In both groups, seven patients (23.3%) were JC virus positive whereas in case group 14 patients (46.7%) and in control group 9 patients (30%) were BK virus positive. There were no statistical significant difference between case and control groups for both JC and BK virus infection rate.

CONCLUSIONS: We concluded that JC and BK virus infection is very prevalent in the first 2 years after transplant and might be monitored appropriately.

KEYWORDS: Polyomavirus, BK virus, JC virus, renal transplantation, PCR.

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Kidney transplantation has become the treatment of choice for patients with end-stage renal disease (ESRD). This has resulted in continued growing number of patients living with a functioning kidney allograft as a percentage of the total ESRD population.¹ After transplantation, immunosuppressant toxicity, and infection are the chief concerns for both patients and physicians. One of

the leading causes of graft loss after kidney transplantation is polyomavirus.²

The two human polyoma species, JC and BK, were isolated from patients with the same initials in 1971. Polyoma virus can cause interstitial nephritis and lead to graft failure in renal transplant recipients. For JC, most persons become seropositive by the age of 10; for BK, the majority of persons are seropositive by 5.

1- Associate Professor, Isfahan Kidney Diseases Research Center, Internal Medicine Department, Isfahan University of Medical Sciences Isfahan, Iran.

2- Associated Professor, Department of Biology, Jahrom branch, Islamic Azad University, Jahrom, Iran.

3- Master of Science, Department of Biology, Jahrom branch, Islamic Azad University, Jahrom, Iran.

4- Medical Laboratory Doctor, Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Science, Isfahan, Iran.

5- Assistant Professor, Isfahan Kidney Diseases Research Center, Internal Medicine Department, Isfahan University of Medical Sciences, Isfahan, Iran.

Corresponding Author: Shahram Taheri
E-mail: shah63tah@yahoo.com

By adulthood, 70-90% of individuals have antibodies to both JCV and BKV. The viruses contain a seroprevalence rate of about 50% for JC and about 90% for BK virus in the age group of 9-11 years.^{3,4} BK virus -related nephropathy was detected in about 8% of renal allograft recipients with loss of renal allograft ranging from 10% to 80% of cases.⁵ Only a few cases of nephritis have been credited to JCV, and now limited information is available with respect to JCV replication in kidney transplant patients and its impact on graft function.⁶ Studies showed that 1 to 7% of renal transplant recipients developed polyomavirus nephropathy,^{7,8} and the incidence appears to be growing.⁹ More than 45% of renal transplant recipients who develop BK nephropathy lose their grafts due to infection.¹⁰ Also, as reported in a clinical study, graft dysfunction attributed to viral infection occurred in 38% of patients with BKV shedding and no patients with JCV shedding.⁶ A study by Costa et al. showed that BKV was positive in 32 of 109 patients (29.6%) and JCV in 20 of 109 patients (18.3%) with renal allograft.¹¹

The number of patients with ESRD on the waiting list for a renal transplant has continued to increase at a steady rate. Any attempts to prolong kidney survival may improve this situation. Polyomavirus have emerged as an important cause of renal allograft failure. There are no agreement guidelines for the detection, prevention, and treatment of polyomavirus infection in transplant recipients. But, screening for polyomavirus is recommended at centers that have experienced high rates of polyomavirus infection.¹² The aim of this study was to compare the frequency of BKV and JCV infections in renal transplant recipients with and without graft dysfunction.

Methods

We performed a case-control study during (Sept 2009 - March 2010) to estimate the frequency of BKV and JCV virus in renal transplant recipients. We enrolled 30 first kidney transplant patients as case group who were hospitalized for renal allograft failure, aged 18

years or older at 1 to 24 months post-transplant time in Al-Zahra hospital in Isfahan University of Medical Sciences, and compared with a control group consisted of 30 first renal transplant recipients that group-matched for age, transplant date and immunosuppressive regimen without graft dysfunction. Ethical approval was obtained from the local research ethics committee in school of medicine before recruitment. Informed consent was obtained from all patients before entering into the study.

In all patients, data obtained included recipient demographic characteristics, immunosuppressive regimen (the immunosuppressive regimen in two groups after transplantation included induction with methylprednisolone and maintenance triple therapy with prednisolone, cyclosporine and mycophenolate mofetil), duration of dialysis, time after transplant and laboratory data including serum creatinine level. For each patient, first morning urinary sample was obtained and evaluated for BK and JC virus detection in our laboratory.

BKV and JCV were extracted from urine samples with QIAamp DNA Mini Kit and quantified by real-time polymerase chain reaction.¹³ Virus copies more than 100 copies/ml considered to be positive. Figure 1 shows the result of JC and BK virus detection by polymerase chain reaction by visualization of bands of the appropriate size on agarose gels after electrophoresis.

Statistical analyses were done with the use of SPSS software (version 16). Chi-square test with Yates' correction, Student t-test and Mann-Whitney U test were used as indicated. P value of less than 0.05 was regarded as statistically significant.

Results

There were 30 patients in both case and control groups. Table 1 shows the demographic, laboratory and clinical characteristics of the study population. There were no significant differences between the cases and controls for age, sex, time post-transplant, immunosuppressive regimen and duration on dialysis. There was no significant difference between groups for

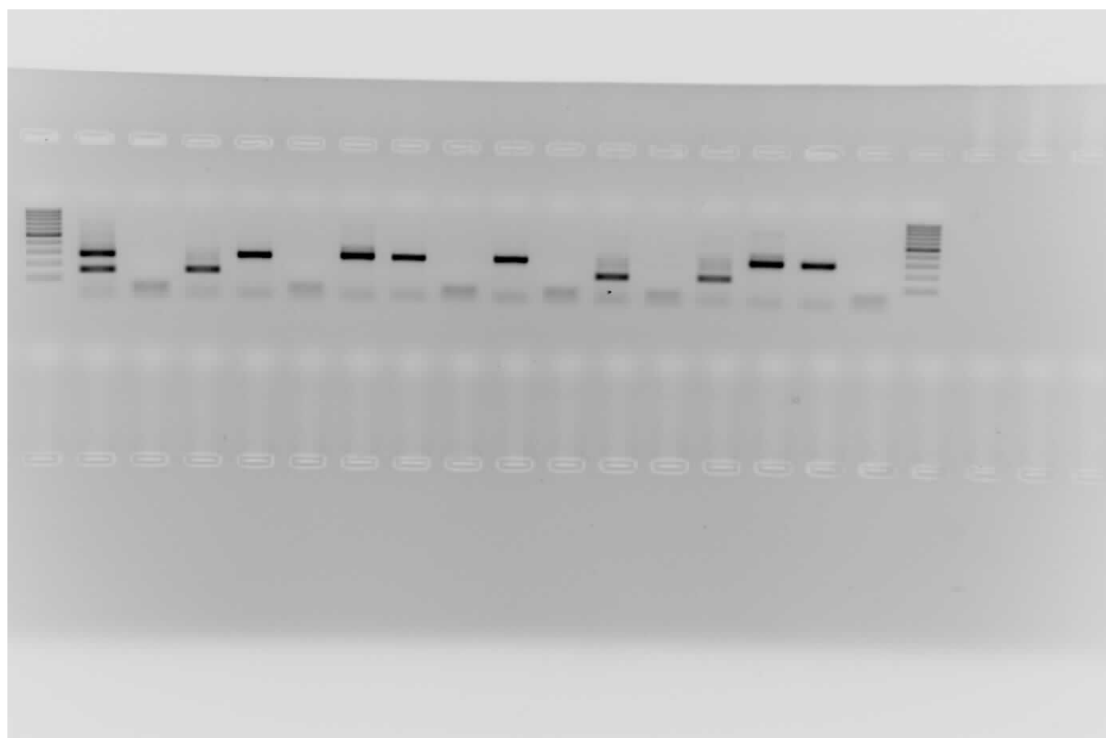


Figure 1. 100-base-pair ladder. The first column was marker 100-base-pair. The second column was positive control. Top of the column was related to BKV and beneath of the column was related to JCV. The third column was negative control. Other columns: BKV was positive if the top of the columns was bold and JCV was positive if the beneath of the columns was bold. The last column was marker 100-base-pair.

Table 1. Demographic, clinical and laboratory characteristics in 60 renal transplant recipients

	Cases (n=30)	Controls (n=30)	P value*
Age (years)	41.4 ± 14.7	46.3 ± 15.2	0.3
Sex			
Female	8 (26.7)	16 (53.3)	0.07
Male	22 (73.3)	14 (46.7)	
Time post-transplant (month)	10.5 ± 7.1	13.2 ± 5.9	0.1
Duration on dialysis (month)	15.9 ± 15.2	16.5 ± 16.1	0.9
BUN(mg/dl)	41.8 ± 13.8	30.9 ± 10.7	0.002
Cr(mg/dl)	2.2 ± 0.7	1.2 ± 0.2	<0.0001
Hb(g/dl)	11.5 ± 3.2	13.4 ± 1.8	0.008
Cause of renal failure			
Diabetes	8 (26.7)	6 (20)	0.8
Hypertension	8 (26.7)	8 (26.7)	
Genetic	4 (13.3)	2 (6.7)	
Other	3(10)	4 (13.3)	
Unknown	7(23.3)	10(33.3)	

Data are mean ± 1 SD and numbers (%). Cases included renal transplant recipients with loss of renal allograft and controls included renal transplant recipients.

* Independent samples t-test and Chi-square test with Yates' correction were used for p values.

immunosuppressive regimen. As shown in Table 1, the mean serum creatinine, BUN and Hb were significantly different between groups ($P < 0.05$, t test).

In the study population, 14/60 (23.3%) patients (case group, seven; control group, seven) were positive for JC virus and 23/60 (38.3%) patients (case group, fourteen; control group, nine) were positive for BK virus. No significant difference was observed between case and control groups of either virus. Results are summarized in Table 2. JCV was detected alone in 13 patients (case group, six; control group, seven); BKV was detected alone in 22 patients (case group, 13; control group, 9); both JCV and BKV just were detectable in one patient in case group.

As shown in Table 3, the Mann-Whitney U test revealed that the median post-transplant time in patients with acute deterioration of their kidney function was significantly shorter in JCV positive than in JCV negative patients [4 (3.7-5.5) vs. 10 (6-19) months], ($P = 0.003$). Also, within controls the median age were 60 (45-61) years in patients with JCV positive and 40 (29-57) years in patients with JCV negative; this difference was also statistically significant ($P = 0.04$).

Discussion

In the current study, the frequency of BKV was 46.7% in cases and 30% in controls. The frequency of JCV was 23.3% in both groups. Post-transplant time in case group was significantly shorter in JCV positive than in JCV negative patients. This may indicate that in these patients, JCV infection may predispose to earlier graft dysfunction. In control group with normal allograft function, JCV was found in older patients.

Frequency of JCV in our study (23.3%) was similar to that reported in other studies, ranging from 13.7 to 36.8%,^{6,11,14,15} and occurred at a higher frequency in comparison to López et al. study.¹⁶ Also Frequency of BKV (38.3%) was higher in comparison to 6.8¹⁵, 18.3¹¹, 13.1%¹⁷ and lower in comparison to 56.3%⁶ but was similar to López et al. study 40.7%.¹⁶ This could be attributable to different study designs and follow-up sampling timings.

In accordance with Drachenberg et al.⁶ the present study showed that in kidney transplant recipients (control group), the frequency of JCV in older patients was more than in younger ones. Some studies showed that whenever JC virus has been identified in the kidney of a patient with interstitial nephritis, BK virus has also been identified and JC virus alone has not been shown to cause interstitial nephritis.^{18,19} But in the present study, JC and BK virus has been identified in one patient. Different methodology and small sample size may be the possible reasons. Muller et al.²⁰ found urinary JC virus excretion in 32% of renal transplant recipients which was in accordance with present study (23.3%). However, no correlation with allograft nephropathy was recognized. The role of JC virus in polyomavirus-associated nephropathy remains unclear, although evidence for JC virus participation in virus nephropathy of renal transplant recipients was previously observed.¹⁹

Ramos et al.²¹ demonstrated that patients diagnosed with polyomavirus presented with variable deterioration of graft function manifested by an increased serum creatinine. That was inconsistent with our results which showed there was no association between serum creatinine with the detection of polyomavirus.

Table 2. Results of polyomaviruses BKV and JCV detection in urine samples from 60 renal transplant recipients

	Cases (n=30)	Controls (n=30)	P value*
JCV(+)	7 (23.3)	7 (23.3)	0.8
BKV(+)	14 (46.7)	9 (30)	0.3

Data are numbers (%). Cases included renal transplant recipients with loss of renal allograft and controls included renal transplant recipients.

*Chi-square test with Yates' correction was used for p values.

Table 3. Analysis of post-transplant time and patients' age as risk factors for polyomavirus in 60 renal transplant recipients

	n	Age (years)	P *	Time post-transplant (month)	P *
Cases (n=30)					
JCV(+)	7	30 [21-46]	NS	4 [3.7-5.5]	0.003
JCV(-)	23	48 [32-57]		10 [6-19]	
BKV(+)	16	44 [28.7-56.2]	NS	9.5 [6-14.2]	NS
BKV(-)	14	43 [26-56.7]		6 [14-17]	
Controls (n=30)					
JCV(+)	7	60 [45-61]	0.04	9 [7-14]	NS
JCV(-)	23	40 [29-57]		13 [9-20]	
BKV(+)	16	54.5 [42-61.7]	NS	12.5 [10.5-20]	NS
BKV(-)	14	42.5 [29-60]		11.5 [8-16.2]	

Data are medians (IQR). Cases included renal transplant recipients with loss of renal allograft and controls included renal transplant recipients. NS, not significant

* Mann-Whitney U test was used for p values.

Cheng XS et al. detected BKV and JCV in the urine samples of 35% and 16% of their patients, respectively, which was nearly the same as 46.7% and 23.3% in our case group and 30% and 23.3% in our control group, respectively.²² They also followed co-infection of both viruses and concluded that infection with either polyomavirus decreases the chance of infection with the other. In our study, also co-infection of both viruses was found only in one patient in the case group.

Saundh BK and coworkers, in the retrospective analysis of urine and plasma samples from 30 renal transplant patients found that eight patients (26.7%) were positive for BK viruria and five patients (16.7%) were positive for JC viruria. Rise in BKV and JCV antibody titers had association with high levels of viruria.

Although the number was similar to some cited studies,^{14,16,21,23} one limitation of the present study was the small number of patients enrolled. The other was lack of confirmation of the nephropathy by kidney biopsy because of unapproved protocol biopsy in our center. Some of our patients in case group

were biopsied but it was not enough to make any conclusion that the renal failure is due to polyoma virus nephropathy.

Long-term studies with enough sample size are needed to define more information about the role of polyomavirus on graft function in kidney transplant recipients. Also, according to the other studies^{12,24,25} and our results, because of risk for this infections, screening during the first 24 months post-transplantation seems to be reasonable.

Conclusion

In conclusion, results of the present study demonstrated that more attention should be paid to polyomavirus infection in renal transplantation receptions.

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Conflict of Interests

Authors have no conflict of interests.

Authors' Contributions

ST, MY, and FK designed the study. ST, MS, MM, SH, SS, AA gathered the data. ST and MS analyzed data. All authors read and approved the final manuscript.

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