
Original article**Prevalence of BK virus in renal allograft recipients pre and post transplantation in Iran****Ali Reza Samarbasf-Zadeh¹, Manoochehr Makvandi¹, Gholamabbas Kayedani¹, Heshmatoallah Shahbazian², Vahid Poorfarziani³, Ramin Yaghoobi⁴, Seyed Mahmoud Latifi⁵**¹*Department of Medical Microbiology, School of Medicine, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*²*Department of Internal Medicine, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*³*Department of Internal Medicine, School of Medicine, University of Baghiatillah, Tehran, Iran*⁴*Research Center of Organ Transplantation, Namazi Hospital, Shiraz University of Medical Science, Shiraz, Iran*⁵*Department of Epidemiology, School of Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran***Received:** May 2009**Accepted:** June 2009

Abstract

BK virus, a member of polyomaviridae, infects humans asymptotically in their early infancy and remains latent in the kidney and urinary tract cells. Reactivation of BK virus in renal-transplanted patients occurs frequently and sometimes leads to nephropathy and rejection of grafted organ. In this study, prevalence of this virus in renal allograft recipients is discussed before and after transplantation. This cross-sectional study performed on 78 renal recipient cases. Blood and urine samples of patients were collected three times; before graft, one month following operation and four months after receiving the graft. All the patients were taking similar drug regimen. The extracted DNA of the samples was investigated by double PCR and semi-nested-PCR. PCR results were analyzed by SPSS software version 11.5 and Mac Nemar test. Out of 78 samples collected from kidney transplanted cases, five urine specimens (6.4%) were positive for BK virus, 10 urine samples (12.8%) turned out as positive for this virus one month after operation and 30 of urine specimens (38.5%) became positive for BK virus four months post-transplantation. Of 78 plasma samples of the same cases, none of them was positive for BK virus before transplantation. One plasma specimen (1.3%) turned out as positive for BK virus and 16 plasma samples (20.5%) were positive for this virus four months after grafted kidney. The difference between the results of the urine samples collected before and four months after kidney transplantation from the patients, was statistically significant ($P<0.05$). Based on the gleaned data it is concluded that BK virus infection increased in a considerable fraction of kidney transplant patients. Therefore, investigation for reactivation of BK virus is recommended in these patients after kidney graft.

Keywords: BK virus reactivation, Semi-nested PCR, Kidney transplantation

Introduction

Human BK virus a member of polyomaviridae was discovered in 1971 in the urine of a 39-old male four months after his renal transplantation following urinary tract obstruction and kidney failure [1]. Primary infection with BK virus occurs during childhood, but the virus can remain latent in kidney and urinary tract system cells for the rest of life [2]. It is estimated that 60-90% of adults have anti-BK virus antibody in their blood and impairment of immune system for any reason like pregnancy or organ transplantation might reactivate this virus [3]. Reactivation of latent BK virus might occur in 10-60% of the renal graft patients and this reactivation is responsible for 1-5% of nephropathy of such cases. Half of the grafted kidneys are rejected following nephropathy [4].

Although the risk factors involved triggering nephropathy by BK virus are not well understood, administration of immunosuppressive drugs, process of transplantation, and the virus by itself are the main suspected risk factors [5]. Therefore, rapid detection of reactivated BK virus in renal-transplanted recipients is crucial for taking appropriate care and treatment strategies to assist reduction of nephropathy and subsequent organ rejection in these cases. Gold standard for detection of reactivated BK virus following nephropathy implies biopsy of kidney. This method is an invasive method, but PCR of BK virus in the urine or blood of this group of patients is a non-invasive alternative. In this study, we report that for rapid detection of prevalence of BK virus, PCR of plasma and urine of renal-transplanted patients is a reliable non-invasive method readily applicable in the diagnostic labs.

Materials and methods

This cross-sectional study performed on 78 renal allograft recipients from three major renal transplant centres in Iran (Tehran

Baghiatollah hospital, Shiraz Research Center of Transplant Studies and Ahvaz Golestan hospital). The study was performed during October 2005 to August 2007 on 51 (65.4%) males and 27 (34.6%) females aged between 16 and 60 (mean 34.87). A consent letter was signed by the patients. From each case, 5ml whole blood in EDTA and 10-50ml urine were taken and collected three times, urine and plasma of the cases before graft operation as negative controls, similar samples one month and four months post-operation. Urine samples were centrifuged at 1500rpm, their supernatants were emptied, and the sediments were kept separately at -70°C until experiment. Blood specimens were centrifuged at 3000rpm and their plasma was saved at -70°C until DNA extraction.

Extraction of DNA was performed with three different methods, Qiagen kit (USA), Cinnage (Iran) and phenol-chloroform extraction described by Sambrook *et al.* [6]. Extracted DNA was subjected to two different PCR sets. In first set of PCR, the primers were PEP-1 and PEP-2 and the size of amplicon was 176bp [7]. The sequences of the primers were as PEP-1: 5'- AGT CTT TAG GGT CTT CTA CC-3' and PEP-2: 5'-GGT GCC AAC CTA TGG AAC AG-3'. For each 25µl PCR reaction, 1µl 2mM MgCl₂, 0.25µl of each primers (100pmol), 2.5µl 10x PCR buffer, 0.5µl dNTPs mix (10 mM) Roche (Germany), 0.15µl *Taq* DNA polymerase (5 units) Cinnagen (Iran), 2.5µl extracted DNA as template and 17.85µl ddH₂O was added.

PCR program was as follow; 1 cycle 94°C for four minutes followed by 40 cycles, 94°C one minute, 55°C one minute and 72°C 1.5 minute. Final extension was performed at 72°C for six minutes. Thermal cycler machine was iCYCLER Bio-Rad (USA). 5µl of PCR product was mixed with 6x loading dye and loaded onto 3% w/v agarose gel LE, Roche (Germany) and after

electrophoresis (130 volts for 80 minutes, 0.5x TBE buffer), the gel was stained in Ethidium bromide (10 μ g/ml), the amplicon was visualized and its size was determined under UV transilluminator Lambert (France) (Fig. 1,2). In each set of PCR, ddH₂O was included as negative control and DNA of BK virus obtained from Shiraz Namazy hospital as positive control. The DNA of all the samples (positive and negative) was subjected to another round of PCR (nested-PCR) for further confirmation of the results of previous PCR.

The sequences of the primers for nested-PCR were as references 4 and 8. (BKJC Out-1: 5'-AAG TCT TTA GGG TCT TCT AC-3' and BKJC Out-2: 5'- GTG CCA ACC TAT GGA ACA GA-3'). The size of first round of PCR with this pair of primers was 176 bp, and 1 μ l of this product was used as template of nested-PCR. The sequences of primers of nested-PCR were as, BKJC out-1: 5'-AAG TCT TTA GGG TCT TCT AC-3' and BKIN: 5'- GAG TCC TGG TGG AGT TCC-3'. Nested-PCR product size was 149 bp. The data were analyzed by SPSS version 11.5 and Mac Nemar test.

Results

Urine samples

Out of 78 urine specimens collected before renal allograft operation, five samples (6.8%) were positive for BK virus. One month after operation, in 10 cases (12.8%) DNA of BK virus was found by PCR and in the samples collected four months post-operation; DNA of BK virus was detectable in 30 specimens (38.5%) by PCR method. The difference between the results of the samples collected before and four months after operation was statistically significant ($P<0.05$) (Table 1). The differences between BK virus positivity of collected samples one

month and four months after operation were statistically significant ($P<0.05$).

Plasma samples

DNA of BK virus was not detectable in all plasma samples collected before kidney transplant operation. After one-month post operation, one plasma sample (1.3%) turned out as positive for BK virus DNA by PCR but after four months, 16 plasma specimens (20.5%) became positive for DNA of BK virus. The difference of the results of pre and four months post-operation, statistically was significant ($P<0.05$) for plasma samples (Table 2). The results of PCR of plasma samples collected one month and four months after transplantation statistically were significant ($P<0.05$) whereas the age of the patients was not a significant factor ($P=0.625$). Extraction of BK virus DNA of positive samples, was performed by three different methods; CinnaGen kit (Iran), Qiagen (USA) and phenol-chloroform method [6]. Only PCR of the template extracted by phenol-chloroform method yielded product. Therefore, for all samples viral DNA was extracted by phenol-chloroform method.

In this study, the results of PCR with PEP-1 and 2 were confirmed by semi-nested PCR. PCR result of three cases that were negative with PEP primers turned positive with nested PCR. Since PEP primers are common for both JC and BK virus, we did not expect more positive case after nested PCR in comparison with PEP primers. It is noteworthy that, a 19 year old male patient, whose urine and plasma samples in 1 and 4 months post-operation were positive for DNA of BK virus suffered from severe kidney failure and hemorrhagic cystitis six months after graft operation.

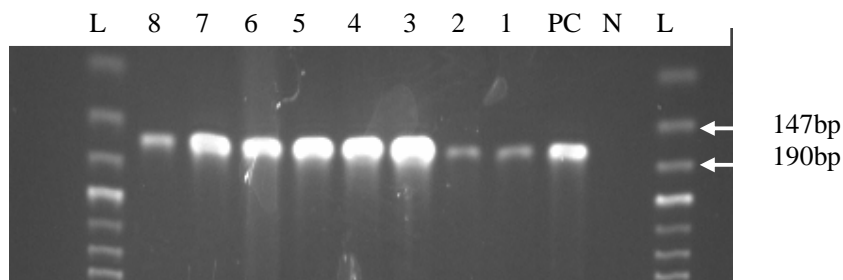


Fig. 1: Agarose gel electrophoresis of BK virus PCR products with PEP primers Track L is Roche DNA ladder. Track N was loaded by negative control. Line PC demonstrates a positive control and lines 1- 8 show positive samples

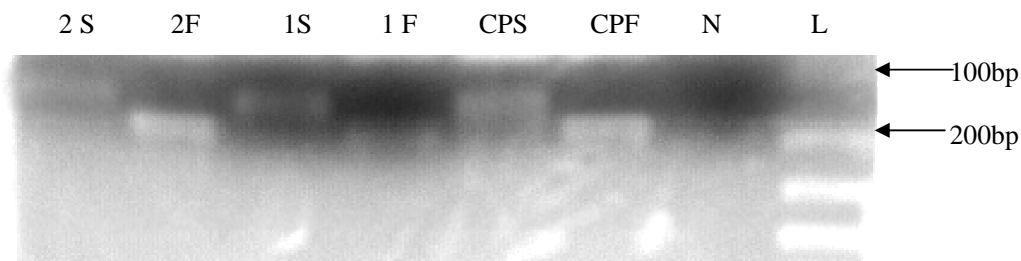


Fig. 2: Agarose gel electrophoresis of 1st and 2nd round of BK virus nested-PCR products Track L was loaded by DNA ladder, First and second round of positive sample is shown by PCF and PCS, tracks 1 and 2 were loaded with 1st (F) and 2nd (S) round of PCR products

Table 1: Comparison of the results of urine samples before kidney graft operation, one month, and four months post-operation for the existence DNA of BK virus

	Positive samples (%)	Negative samples (%)	Total (%)	P value
Pre-operation	5 (6.8%)	73 (93.2%)	78 (100%)	-
One month post-operation	10 (12.8%)	68 (87.2%)	78 (100%)	0.063
Four months post-operation	30 (38.5%)	48 (61.5%)	78 (100%)	.000

Table 2: Comparison of the results of plasma samples before kidney graft operation, one month, and four months post-operation for the existence DNA of BK virus

	Positive samples (%)	Negative samples (%)	Total (%)	P value
Pre-operation	0 (0%)	78 (100%)	78 (100%)	-
One month post-operation	1 (1.3%)	77 (98.7%)	78 (100%)	0.74
Four months post-operation	16 (20.5%)	62 (79.5%)	78 (100%)	0.00

Discussion

To determine reactivation of persistent BK virus, mRNA of virus capsid protein needs to be extracted and the cDNA must be used as template of PCR. In this project, only prevalence of BK virus was determined in three different period of time in transplant patients referred to three major university teaching hospitals. Although in this report reactivation of BK virus has not been investigated, prevalence of this virus scaled up in the last sampling in kidney-grafted cases. The reports show that in 10-60% of transplanted patients, reactivation of BK virus occurs and in 1-5% of this percentage, the reactivation ends up to nephropathy. Moreover, in half of nephropathic cases, BK reactivation is the cause of rejection of transplanted kidney [2-4]. Hence, determination of reactivated BK virus in kidney recipients is of great importance following operation.

Of the 78 collected urine and plasma samples, 38.5% of the urine and 20.5% of plasma specimens were positive for BK virus. The difference between urine and plasma samples was statistically significant. These figures are in accordance with other reports. In Switzerland, Hirsch *et al.* [8] found that 30% of urine samples and 13% of plasma samples collected from kidney recipients were positive for BK virus. In France, 57% of urine and 29% of plasma specimens of this kind of patients have been positive for BK virus [9]. Almost similar result has been reported from Spain where 33% of urine and 7.5% of plasma specimens were positive for BK virus [10]. In a similar study in Kuwait, Nampoory *et al.* [11] have found that 45% of urine and 26.1% of plasma samples were BK virus positive.

For the first few samples, extraction of DNA was performed by phenol-chloroform parallel with two commercial extraction kits. Following PCR amplification, the template extracted with one of the kit did not yield any PCR product and for the other kit only a faint band was visible in the gel. We found that phenol-chloroform extraction was the

most sensitive method for extraction of BK virus DNA since the PCR products had a sharp visible band in agarose gel and extraction of the remaining samples was performed by this method. PCR of none of the specimens collected one month before kidney operation was positive for BK virus and almost all positive results belonged to the samples collected four months after operation. As the results of tables 1 and 2 show, there is a time lag between urine BK virus positivity and viremia by this virus. All templates were amplified with three different sets of primers; PEP and semi-nested PCR primers. As the result shows, semi-nested PCR is more sensitive than one round PCR with PEP primers.

Conclusion

Following kidney graft, BK virus is reactivated in some cases.

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