Prevalence of BK virus in kidney transplant recipients in Albania

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Abstract

Aims: BK virus (BKV) is a polyomavirus with a circular DNA. Infection with BKV occurs during childhood, with a prevalence in adults from 60% to 100%. Following primary infection, BKV remains latent in the kidneys and can be reactivated in immune deficiency conditions, including transplantations.
Methods: This study was performed on 49 consecutive adult recipients of kidney transplant, who were presented to the hospital during the period 10 June 2012 - 31 December 2013. From each case were taken 5 ml whole blood in a gel tube. Viral DNA from clinical sample was extracted using Bioneer Exiprep 16^{TM} system. Clinical specimens were screened for BKV – DNA by using a Bioneer AccuPower® quantitative RT – PCR Diagnostic Kits.
Results: Among 49 patients, BKV viremia was present in 14.2% (7 patients) with mean load of 1.053043 x 10^6 copy/µl.
Conclusion: In this study, we demonstrated an impact of BKV replication on the renal function of our patients who received kidney transplants. These findings should be regarded with caution though given the lack of follow-up and the absence of renal biopsy specimens.

Keywords: BKV, kidney transplant, nephrology.

Introduction

The human polyomavirus is a small, nonenveloped, DNA virus. Two species have been described as pathogenic in humans: JC virus and BK virus.

BK virus (BKV) is a polyomavirus with a circular DNA. Infection with BKV occurs during childhood and adulthood in a range from 60% to 100%. Following primary infection, BKV remains latent in the kidneys and can be reactivated in immune deficiency conditions, including transplantations (1-5). The prevalence of BK virus-associated nephropathy (BKVAN) has increased from 1% to 10% in the past decade. BK virus has been associated with hemorrhagic cystitis in bone marrow transplant patients, as well as with ureteral stenosis and transplant-associated nephropathy in patients receiving kidney transplants. Because BKVAN patients show progressive allograft dysfunction without specific symptoms, it is difficult to differentiate from acute graft rejection (6-9).

There are some methods such as discovery of decoy cell of BKV in urine by electron microscopy or PCR that are of limited utility in clinical use.

The quantification of BK virus in blood of renal transplant patients is useful for diagnosing BK virus nephropathy and also for monitoring the response to therapy.

Methods

This study was performed on 49 consecutive adult recipients of kidney transplant, who showed up at the University Hospital Center "Mother Teresa" in Tirana between 10 June 2012 and 31 December 2013. Twenty six (53.1%) of these 49 patients were males and 23 (46.9%) were females. All patients were aged 40±10 years. The samples were obtained with a median of 45 days after transplantation. Characteristics of the study population included in this study are presented in Table 1.

Table 1. Characteristics of individuals included in this study

General characteristics	
Organ transplanted	Kidney
Study period	From 10 June 2012 to 31 December 2013
Total number of patients	49
Age (mean ± SD)	40±10 years
Males	26 (53.06%)
Females	23 (46.94%)
Time of sample collection, median days after transplant	45
Immunosuppressive agents (numbers)	
Mycophenolic acid	32
MMF	15
Tacrolimus	32
Ciclosporine	17
Everolimus	2
Reason for hospital visit (numbers)	
Renal dysfunction	13 of 26
Infection	8 of 16
Other	4 of 7

From each case there were drawn 5 ml of whole blood in a gel tube. Blood specimens were centrifuged at 3000 rpm and their serum was saved at –70^{0}C until DNA extraction was performed.

Viral DNA from clinical sample was extracted using BioneerExiprep 16™ system. BioneerExiPrep 16™ is an automated system, designed for the extraction of viral DNA/RNA from various clinical samples. Viral DNA/RNA is extracted from clinical samples by using a Lysis buffer to disrupt viral structure. The binding buffer and silica magnetic beads bind the exposed Viral DNA/RNA to the surface of the beads. The Washing buffer rinses any impurities that may exist, and the Elution buffer extracts the pure DNA/RNA from the beads.

Detection and amplification of BKV-DNA.

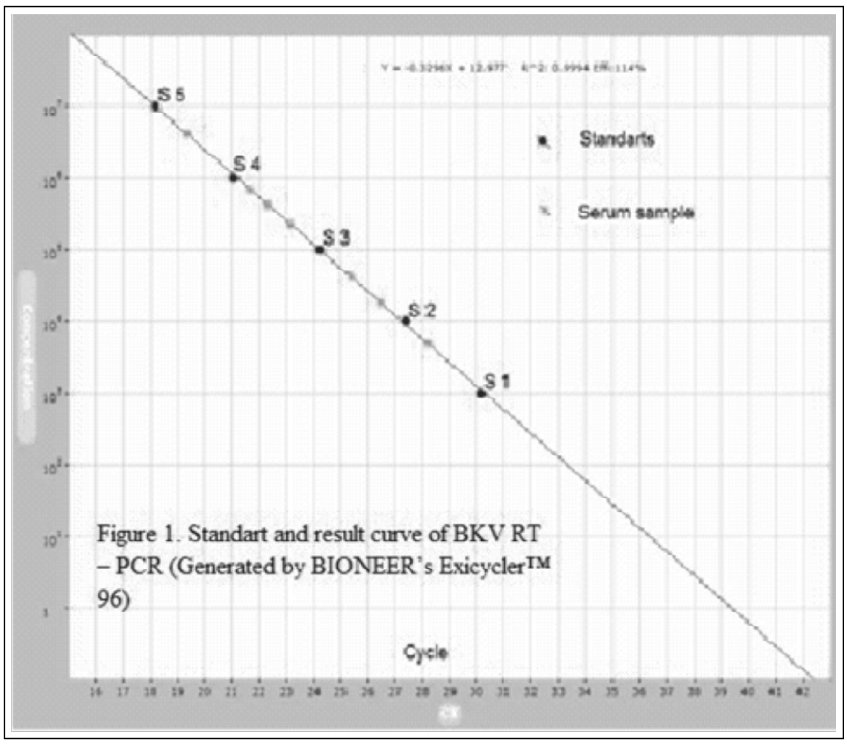
Clinical specimens were screened for BKV – DNA by using a Bioneer AccuPower® quantitative RT – PCR Diagnostic Kits. The kit was designed to maximize reproducibility and ease-of-use by vacuum-drying all reagents for PCR including primers, probes, DNA polymerase, dNTPs and salts. The primer-probe set was selected from a pool of primer-probe combinations designed by bioinformatics algorithms to achieve maximized amplification efficiency. This kit is optimized for use with BIONEER's Exicycler™ 96. Data was analyzed through 'ExiDiagnosis Analysis' program.

Univariate analysis was used to assess factors associated with impairment of renal function. In all cases, a p-value ≤ 0.05 was considered as statistically significant. Statistical analysis was performed with SPSS, version 20.0.

Results

Among 49 patients, BKV viremia was present in 14.2% of them (7 patients), with a mean load of 1.053043 x 10^6 copy/µl. Results of BKV RT – PCR are presented in Figure 1.

Figure 1. Standard and result curve of BKV RT – PCR
(generated by BIONEER's Exicycler™ 96)



Relationships between BKV replication and characteristics of recipients are presented in Table 2.

Table 2. Relationships between BKV replication and characteristics of recipients

Characteristic	Total no. of patients	No. of patients without BKV Viremia present	No. of patients with BKV Viremia present
Kidney transplant	49	42	7
Age mean	40 ± 10	40 ± 10	40 ± 10
Immunosupresive agent			
Mycophenolic acid	29	18	5
MMF	18	8	3
Tacrolimus	27	25	4
Ciclosporine	16	12	5
Everolimus	3	2	-

On the average, viremia was detected 192 days after kidney transplantation. Recipients of kidney transplants had high levels of creatinine when viremia was present.

Table 3 displays the connection between BKV replication and clinical characteristics of recipients.

Table 3. The connection between BKV replication and clinical characteristics of recipients

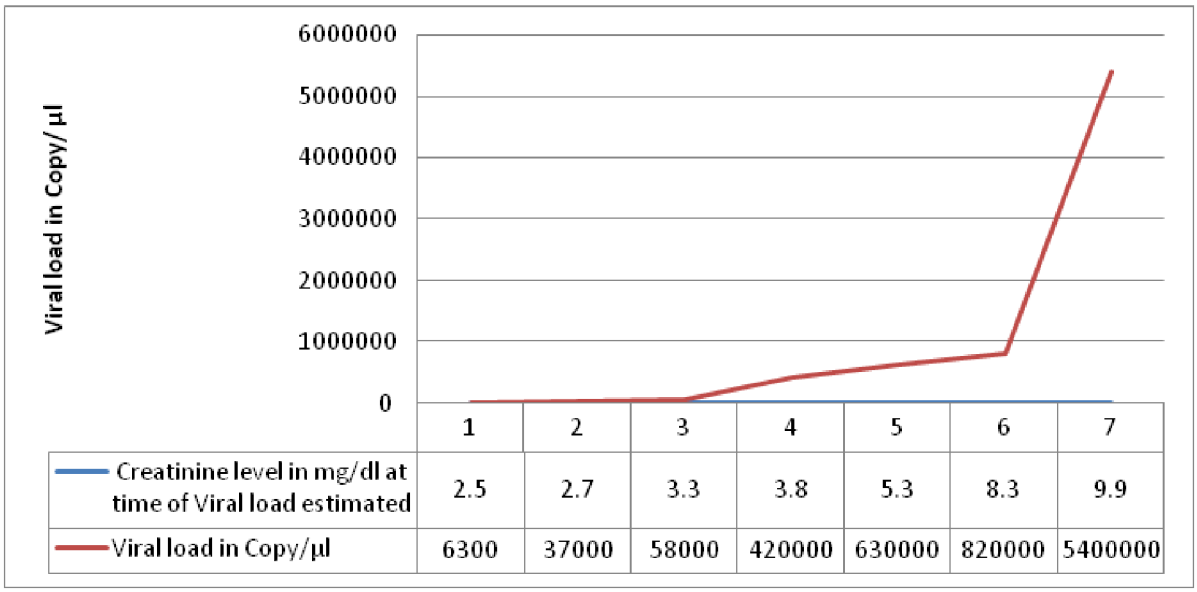
	Total no. of patients	No. of patients without BKV Viremia present	No. of patients with BKV Viremia present
Kidney transplant	49	42	7
Hematuria	49	6	2
Creatinine level			
>1,5 mg/dl	49	4	7
<1,5 mg/dl	49	38	0

In the course of the study, we found that the rate of deterioration graphs expressed by creatinine level was proportional to the level of viral replication expressed by the viral load in the blood. Relationships between creatinine level and viral load in the blood are exhibited in Table 4 and graphically shown in Figure 2.

Table 4. Creatinine level in mg/dl at the time of viral load estimates

	Viral load in Copy/µl
2.5	6300
2.7	37000
3.3	58000
3.8	420000
5.3	630000
8.3	820000
9.9	5400000

Figure 2. Relationship between creatinine levels and viral load



	1	2	3	4	5	6	7
Creatinine level in mg/dl at time of Viral load estimated	2.5	2.7	3.3	3.8	5.3	8.3	9.9
Viral load in Copy/µl	6300	37000	58000	420000	630000	820000	5400000

Discussion

The diagnosis and the severity of BKV infection correspond of the pathogenesis of BKV nephropathy. Viral replication begins early after transplantation and has three stages: viruria, subsequently viremia and, next, nephropathy (10).

Viruria can be detected by PCR for BKV-DNA. This test that discovers BKV infections has high sensitivity but low specificity for nephropathy because the virus could have an origin in the urinary tract (11,12). Hence, finding BKV DNA in the plasma or viremia is always a good indicator of nephropathy. When the infection intensifies, the replication increases.

Our study shows BKV replication in kidney transplant recipients and its relation with bad renal function. In patients with kidney transplantation the prevalence of BKV in our study was 14.2%, which is similar to other studies that show the same values approximately in the range of 13%-68%.

The clinical significance of BKV viruria and viremia in non-renal function is almost unknown. In our study, the relation between the use of tacrolimus with mycophenolate and BKV viremia was not associated with renal dysfunction or with BKV replication. In our study, we can conclude that replication of BKV has an independent impact on the renal function of patients who received kidney transplants because samples were obtained at a time of 192 days after transplantation. At this time renal dysfunction is multi-factorial.

Our study has some limitations, because it did not include a follow-up sampling of patients with BKV viruria, and also lack the renal biopsy specimens. Furthermore, this study does not show whether immunosuppression should be decreased in a recipient of a non-renal transplant with BKV discovered in blood and/or urine.

Nonetheless, in this study we demonstrated an impact of BKV replication on the renal function of our patients in Tirana who received kidney transplants. In conclusion, regular detection in first stages is needed for new therapeutic strategies of patients undergoing renal transplantation.

Conflicts of interest: None declared.

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