

Comparative Evaluation of In-House and Commercial Real-Time PCR Methods for the Detection of the BK and JC Viruses in Clinical Samples

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Abstract

Aim The two most common human polyomaviruses are the BK (BKV) and JC viruses (JCV). Diseases associated with polyomavirus usually occur in cases of severe cellular immunosuppression. BKV and JCV can cause many diseases, especially if they are reactivated in an immunosuppressed host. The aim of this study is to compare and evaluate the results of real-time polymerase chain reaction (PCR) methods targeting the small and large T gene regions of the viral genome, considering polymorphisms occurring in the viral genome of BKV and JCV.

Materials and Methods Urinary specimens of 82 patients were taken from immunosuppressed patient and sent to molecular microbiology laboratory of Meram Medical Faculty. The small t gene was investigated using a commercial kit (LightMix, Roche) by real-time PCR method. Large T gene was investigated by using the optimized in-house real-time PCR method. Sequence analysis was accepted as the standard method.

Results BKV positivity was detected in 9 samples and JCV positivity in 61 samples by real-time PCR method specific to small t gene region; BKV positivity in 21 samples and JCV positivity in 67 samples were determined by real-time PCR method specific to the large T gene region. Statistically, there was a significant difference for BKV, but not significant difference for JCV detection between the two methods.

Conclusion Different polymorphisms in the target gene regions were responsible for the different outcomes obtained from this study. With this sensitivity and specificity, in-house PCR method which we used is a candidate for routine diagnosis.

Keywords

- BK virus
- JC virus
- real-time PCR

Introduction

Human polyomaviruses are double-stranded deoxyribonucleic acid (DNA) viruses, located in the *Polyomaviridae* family.¹ The two most common human polyomaviruses are the BK (BKV) and JC viruses (JCV) that were first described in the 1970s.^{2,3} Diseases associated with polyomavirus usually occur in cases of severe cellular immunosuppression, such

as organ transplantation, acquired immune deficiency syndrome, and leukemia.⁴

Most people encounter BKV and JCV in their childhood; transmission is thought to occur through the respiratory or oral route.⁵ Infection is mild during this period.^{6,7} Subsequently, viruses remain latent in the kidneys and B lymphocytes.^{8,9} BKV and JCV can cause many diseases, especially if they are reactivated in an immunosuppressed



host. BKV has been reported to cause more commonly ureteral stenosis, hemorrhagic cystitis, and nephropathy,¹⁰ and less commonly, pneumonia, retinitis, liver diseases, and meningoencephalitis.¹¹ Approximately 80% of kidney transplant recipients have BKV, and 5 to 10% of them develop BKV-associated nephropathy.^{12,13} JCV may cause latent infections of the kidneys,¹⁴ but neurological infections, especially in immunosuppressed patients.¹⁵

BKV and JCV viral loads can be determined from urine, feces, blood, and cerebrospinal fluid samples.¹⁶⁻¹⁸ The BKV viral load is usually found in the urine, 1,000 times more than that of the plasma.¹⁹ Similarly, JCV has been reported to be excreted mostly in urine in healthy individuals.²⁰ The polymerase chain reaction (PCR) method is nowadays defined as the “gold standard” for the detection of polyomaviruses.²¹ To achieve the best performance in real-time PCR methods, target sequence must be carefully selected from conserved regions and regularly evaluated against new sequence.^{21,22} The BKV and JCV genomes show large sequence variations. These sequence variations are also seen in different subtypes. This makes the primer and probe design used in real-time PCR analyses difficult to detect BKVs and JCVs.²³⁻²⁶

The aim of this investigation was to compare laboratory diagnostic performance of two different real-time PCR methods targeting two conserved regions of the viral genome (small t gene and large T gene regions).

Material and Methods

This study was performed at the Department of Molecular Laboratory of Medical Microbiology, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey. Urinary samples from 82 patients aged over 18 years old, which were sent from the organ transplant clinic and the clinics of the Hematology and Oncology Departments to Molecular Laboratory, on suspicion of BKV and JCV were included in the study.

DNAs of the collected BKV and JCV samples were manually extracted using a readymade commercial extraction kit (High Pure Viral Nucleic Acid Kit, Roche Diagnostic, Germany) according to the manufacturer's instructions. Extracted DNA was stored at -20°C until use. Same DNA extracts were used for both assays.

First, presence of the small t gene region was investigated using a commercial kit (LightMix, Roche, United States) by real-time PCR method. It was aimed to amplify the 175-base pair (bp) region of the small t gene region of BKV and the 172 bp region of JCV. Positive controls and internal control for BKV and JCV were used for each study to assess the performance of the analysis. The data was obtained at the end of the study while read on channel 640 for BKV and on channel 705 for JCV. The cycle threshold (Ct) obtained under 37 for each sample was evaluated as positive according to the manufacturer's instructions.

All samples were also tested for the presence of large T gene region. Primer and probe sequences are presented in **Table 1**.

Table 1 Primers and probes used in the large T gene region-specific real-time PCR method

Primer-probe	Sequence (5'-3')
BKV-F	AGCAGGCAAGRGTCTATTACTAAAT
BKV-R	GARGCAACAGCAGATTCYCAACA
BKV-probe	FAM-AAGACCCTAAAGACTTTCYCTGTATACACCA GTTT-TAMRA
JCV-F	CTAAACACAGCTTGACTGAGGAATG
JCV-R	CATTTAATGAGAAGTGGGATGAAGAC
JCV-probe	FAM-TAGAGTGTGGGATCCTGTGTTTTTCATC ACT-TAMRA

Abbreviation: PCR, polymerase chain reaction.

The lyophilized primers and probes from the MacroGen (Humanizing Genomics, Seoul, South Korea) firm were diluted with nuclease-free water in accordance with the manufacturer's recommendations and made ready for use. A commercial kit (LightCycler 480 Probes Master, Roche Applied Science, Indianapolis, Indiana, United States) was used for the master mixture. For positive controls, BKV and JCV positive controls obtained using the “LightMix Kit Polyomaviruses JC and BK” (Roche Applied Science) were used. The data obtained at the end of the study were read on channel 530 for BKV and JCV. The Ct obtained under 40 for each sample was evaluated as positive.

Statistical Analysis

The results obtained from specific real-time PCR analyses of small t gene and large T gene regions were compared using a chi-square test. In addition, based on patient record information, evaluations of gender and age associations of BKV and JCV infected patients were done by using a chi-square test. GraphPad InStat version 3.10 (GraphPad Software, San Diego, California, United States) was used for all statistical evaluations. *p*-Values under 0.05 were considered statistically significant.

Results

Of the 82 patients whose samples were taken for the study, 48 were male and 34 were female (**Table 2**). Of the patients whose urine samples were taken, 26 were under 50 years old and 56 were over 50 years old. The age of the patients ranged from 19 to 86 years. The mean age was 56.1, so the age 50 was taken as the limit in statistical calculations.

Statistically, in terms of BKV and JCV, there was no significant difference between the gender groups as well as between below 50 years of age and above 50 years of age. In all of the patients (*n* = 82) from whom the samples were taken, there was immune suppression due to underlying hematologic/oncologic disease or organ transplantation.

In detecting BKV, positivity was detected in 9 samples by the first method (with commercial kit) and in 21 samples by the second method (with in-house method) (**Table 3**). There was a statistically significant difference between the

Table 2 The distribution of positivity determined by both methods according to gender and age in BK and JC viruses

Method	BKV				JCV			
	Age		Gender		Age		Gender	
	< 50	> 50	Male	Female	< 50	> 50	Male	Female
Commercial kit (number/positive)	26/5	56/4	48/4	34/5	26/16	56/45	48/39	48/40
In-house method (number/positive)	26/8	56/13	48/12	34/9	26/19	56/48	34/22	34/27

Table 3 BK and JC virus results detected by real-time PCR methods

Results	Commercial kit	In-house method
BK positive	9	21
JC positive	61	67
Negative	12	8
Total	82	82

Abbreviation: PCR, polymerase chain reaction.

two methods ($p = 0.0252$, chi-square test) when the data were compared. In detecting JCV, positivity was detected in 61 samples by the first method and in 67 samples by the second method (► **Table 3**). There was no statistically significant difference between the two methods ($p = 0.3457$, chi-square test) when the data were compared.

After studying by the two methods, 19 samples with different PCR results were studied one more time by both the real-time PCR methods. Similar results were obtained in the second studies as well. Subsequently, a sequence analysis of VP1 regions of these 19 samples was performed using the Sanger method. According to the results of the sequence analyses, 12 samples were compatible with the first method, 4 samples were compatible with the second method, and 3 samples were incompatible with the both methods.

According to the result of sequence analysis, for BKV, the sensitivity, specificity, positive predictive value, and negative predictive value of the commercial kit were 100, 97.3, 77.8, and 100%, respectively. For JCV, its sensitivity, specificity, positive predictive value, and negative predictive value were 89.7, 100, 100, and 66.7%, respectively.

For BKV, the sensitivity, specificity, positive predictive value, and negative predictive value of the in-house PCR were 100, 81.3, 33.3, and 100%, respectively. For in-house JCV, its sensitivity, specificity, positive predictive value, and negative predictive value were 98.5, 100, 100, and 93.3%, respectively (► **Table 4**).

Discussion

Human polyomaviruses are endemic and infect most of the healthy individuals worldwide. In the epidemiological studies conducted, the seroprevalence of these infections varies between 35 and 90%.^{27,28} BKV and JCV genetically show approximately 75% nucleotide similarity among themselves.²⁹

BKV is associated with hemorrhagic cystitis, urethral stenosis, and other diseases of the urinary system in patients with immunosuppressive treatment. To be able to treat the infection effectively, it is necessary to detect disease agents early, and determine the presence of polyomaviruses in urine and blood samples accurately. The high frequency of BKV and JCV infections and the high risk of transmission of infection in the hosts who are immunologically impaired have made rapid diagnosis of polyomaviruses an important issue in laboratory diagnosis.

For the management of the disease, it is important to define the polyomavirus. Conventional methods, such as virus isolation and serological analysis, are not widely used in the routine diagnosis of polyomaviruses today due to reasons of technical limitations or commercial reagent deficiency. Nucleic acid amplification techniques, especially PCR, have superior sensitivity in detecting polyomaviruses than other diagnostic methods.³⁰ Up to date, different real-time PCR methods have been developed targeting small t, large T, and VP1 genes. It has been reported that the VP1 gene region is prone to mutation and that the small t and large T gene regions are more similar compared with the virus variants and are more suitable for the detection of polyomaviruses.^{31,32} So far, it has been determined that there is no study comparing the performance of real-time PCR methods targeting small t and large T gene regions in detecting BKVs and JCVs from clinical samples. This situation increases the originality of this study.

Within the scope of our study, we aimed to compare clinical performance of two different real-time PCR methods targeting two conserved regions of the polyomavirus viral genome (small t gene and large T gene regions). For this reason, we used primers and probes designed by Dumoulin and Hirsch,³³ taking into consideration the polymorphisms that have taken place in the large T gene region up until now. We searched for the small t gene, using a commercially available kit. While 10 copies of BKV and JCV DNA can be identified using a commercial kit prepared specifically for the small t gene, 4.15 copies of BKV DNA and 3.37 copies of JCV DNA can be identified using in house method, specific to the large T gene region, defined by Dumoulin and Hirsch.

The differences between the numbers of positive samples obtained by the two methods can be attributed to the fact that the primer pairs used in both the methods target different gene regions and the changes (point mutation, deletion,

Table 4 Sensitivity, specificity, positive predictive, and negative predictive values for BKV and JCV of the commercial kit and in-house method

	BKV		JCV	
	Commercial kit	In-house method	Commercial kit	In-house method
Sensitivity	100	100	89.7	98.5
Specificity	97.3	81.3	100	100
Positive predictive value (%)	77.8	33.3	100	100
Negative predictive value (%)	100	100	66.7	93.3

insertion, substitution, etc.) that occur in these gene regions. These changes can lead to incorrect/different results by inhibiting primer binding.

When we compared the data obtained, we found that there was a statistically significant difference in the detection of BKV between these two methods. This led us to the idea that we needed to prove the correctness of the method we applied. According to the results of the sequence analyses, 12 samples were compatible with the first method, 4 samples were compatible with the second method, and 3 samples were incompatible with both the methods.

In conclusion, due to sensitivity and specificity rates of in-house PCR method, new in-house PCR method can be successfully used in the detection of BKVs and JCVs. So much so that the sensitivity of the in-house method in detecting JCVs was higher than the sensitivity of the method of the commercial kit. This finding also necessitates that commercial real-time PCR kits should update their target gene regions regularly according to newly obtained polymorphism to ensure the optimal performance at diagnosis.

Conflict of Interest

None declared.

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