

## THE PREVALENCE OF HUMAN POLYOMAVIRUSES IN URINE SAMPLES OF IMMUNOCOMPETENT INDIVIDUALS IN THE SERBIAN POPULATION

DANIJELA KARALIĆ, IVANA LAZAREVIĆ, MAJA ĆUPIĆ and TANJA JOVANOVIĆ

*University of Belgrade, Faculty of Medicine, Institute of Microbiology and Immunology, 11000 Belgrade, Serbia*

**Abstract** – The BK (BKV) and JC viruses (JCV) are human polyomaviruses. After primary infection, they persist as latent infection in the kidneys. Immunosuppression leads to their reactivation, which is associated with life-threatening diseases such as polyomavirus-induced nephropathy and progressive multifocal leukoencephalopathy. However, the behavior of these viruses in immunocompetent individuals is still an open question with no right answer. The aim of this study was to determine the prevalence of BKV and JCV shedding in the urine of immunocompetent individuals from the Serbian population. Sixty-five urine samples were collected and tested for the presence of BKV and JCV DNA by PCR. JCV DNA was detected in 19/65 (29.2%) and BKV DNA in 3/65 (4.6%) of the urine samples. Forty-three (66.2%) urine samples of the immunocompetent donors were negative for both viruses. The present study provides the first results of urinary excretion of human polyomaviruses in the Serbian population.

**Key words:** Human polyomaviruses, immunocompetent, urinary excretion

### INTRODUCTION

Human polyomaviruses are members of *Polyomaviridae*. The BK (BKV) and JC (JCV) viruses are the first polyomaviruses isolated and described in 1971. BKV was isolated from the urine of a renal transplant patient who suffered from ureteral stenosis (Gardner et al., 1971) and JCV from the brain tissue of a patient with Hodgkin's lymphoma who developed progressive multifocal leukoencephalopathy (PML) (Padgett et al., 1971).

BKV and JCV are two of the smallest DNA viruses that are composed of non-enveloped, icosahedral capsids with a super-coiled double-stranded DNA genome (Imperiale and Major 2007). The virion consists of 72 pentamers of the major capsid protein VP1, with each pentamer associated with a single copy of the minor capsid proteins VP2 and VP3 (Liddington et al., 1991; Stehle et al., 1996). The

genomes of BKV and JCV are divided into three regions: the early coding region, which encodes the large tumor antigen (TAg) and small tumor antigen (tAg); the late coding region, which encodes the viral capsid proteins VP1, VP2 and VP3, the non-structural agnoprotein; and the non-coding control region (NCCR), which contains the viral promoters and origin of replication (Jiang et al., 2009).

All JCV strains are members of a single serotype (Major, 2001) but sequence analysis of a 215bp DNA sequence at the 5' end of the VP1 major capsid protein gene has led to the identification of eight genotypes and various subtypes (Agostini et al., 2001). The most prevalent types in Europe and the United States are type 1 and type 4 (Agostini et al., 1995). JCV types 3 and 6 are African genotypes (Agostini et al., 1995; Guo et al., 1996) and 2, 7 and 8 dominate in Asia, Japan and Indonesia (Agostini et al., 1995; Jobes et al., 1998). The ubiquitous distribution of JCV combined

with a transmission mechanism largely within families or population (Kunitake et al., 1995; Kato et al., 1997; Suzuki et al., 2002; Zheng et al., 2004), make it attractive candidate for reconstructing human migration dating to prehistoric times (Pavesi, 2005).

BKV is the only primate polyomavirus that has subtypes distinguishable by immunological reactivity (Knowels, 2006). Nucleotide variations in a major capsid protein have been used to classify BKV isolates into subtypes (Jin et al., 1993). Subtype I predominates in all geographical regions, subtype IV occurs at lower rates and subtypes II and III rarely occur. In addition, subtype I has been subdivided into three subgroups called Ia, Ib and Ic based on nucleotide variations within the typing region (Takasaka et al., 2004).

Primary infections with both viruses usually occur during early childhood and are asymptomatic or linked to mild respiratory disease (Goudsmit et al., 1981). The mode of transmission for these viruses is not yet firmly defined but according to the literature, a respiratory route is the most likely. Potential alternative modes of transmission include urino-oral, transplacental and transmission by blood transfusion and organ transplantation (Bofill-Mas et al., 2001; Hirsch and Steiger, 2003). After primary infection, both viruses persist in the kidneys.

Certain factors lead to the reactivation of human polyomaviruses and immunodeficiency seems to be the most important. In immunocompromised individuals (AIDS, cancer patients, transplant organ recipients, etc.), JCV reactivation can cause a fatal brain infection of the oligodendrocytes known as PML (Major et al., 1992; Khalili et al., 2006). On the other hand, reactivation of BKV has been associated with hemorrhagic cystitis, ureteral stenosis and polyomavirus induced nephropathy (Apperley et al., 1987; Gardner et al., 1971). Polyomaviruses JCV and BKV also have the ability to induce tumors in laboratory animals (Imperiale, 2000) and they have been associated with some solid tumors in humans (in particular, brain cancers) (Krynska et al., 1999; Hayashi et al., 2001). When reactivation occurs in

immunocompetent individuals, it leads to asymptomatic viruria (Kitamura et al., 1990).

Epidemiological studies have shown that JCV infection is widespread in the human population, with a seroprevalence rate ranging from 70 to 90% (Padgett and Walker, 1973). Seroconversion for both viruses occurs in childhood, with BKV seropositivity reaching 90% in children aged 5 to 9 and JCV seropositivity reaching 50 to 60% after the age of 10 (Knowels, 2006).

According to the results of studies performed in Europe, the frequency rate of JCV viruria is between 20% and 52% (Agostini et al., 2001; Pagani et al., 2003; Schaffer et al., 2006; Kmiecik et al., 2008) and approximately 5% of immunocompetent individuals have BKV viruria (Doerries et al., 2001). However, there are no data about the frequency rate of JCV and BKV in the Serbian population and therefore the aim of this study was to determine the prevalence of BKV and JCV shedding in the urine of immunocompetent individuals from the Serbian population.

## MATERIALS AND METHODS

### *Urine samples and donors*

Sixty-five urine samples were collected from donors (healthy volunteers or nonimmunocompromised patients) attending the Microbiology Laboratory of the Institute of Microbiology and Immunology for routine analysis of urine. Thirty-seven urine samples were donated by male donors and 28 by female donors. Single urine samples were collected from all individuals and stored at  $-70^{\circ}\text{C}$  until tested. Informed consent was obtained from each donor and the study was approved by the Ethics Committee of Faculty of Medicine, University of Belgrade.

### *DNA extraction and amplification of BKV DNA and JCV DNA*

Ten milliliters of urine samples was centrifuged at 4300g for 10 min. The cell pellet was resuspended in 10ml of sterile phosphate-buffered saline (PBS) and

**Table 1.** Prevalence of BKV and JCV urinary excretion by gender in Serbian population

	No. of patients	BKV positive	JCV positive	Both BKV and JCV negative	Both BKV and JCV positive
<b>Male</b>	39	2	11	26	0
<b>Female</b>	26	1	8	17	0
<b>Total</b>	65	3	19	43	0

re-centrifuged. The supernatant was discarded and viral DNA was then extracted by using a QIAamp DNA Mini Kit according to the manufacturer's instructions (Qiagen, Valencia, CA). Viral DNA was stored at -20°C until used for PCR.

From extracted DNA, the 116bp fragment within the VP2 coding region of the BKV genome was amplified by using primers Fw 5' TGCTCCTCAATGGATGTTGC 3' and ReV 5'attgaggagcagttctt3'. The amplification was run in a total volume of 25 µL containing 12.5µL PCR Master Mix (QIAGEN Taq PCR Master Mix, Hilden Germany) 1µL (1µM) FW and 1µL (1µM) REV primers, 5µL previously isolated DNA and 5.5µL injection grade water.

The PCR program, for amplification of BKV DNA, was initial denaturation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 40 s and extension at 72°C for 1 min with a final extension step of 72°C for 10 min.

A seminested PCR was performed for the amplification of the 495bp fragment within the VP1 coding region of the JCV genome. The first PCR round was done with the primer pair P13 (Fw 5' TTCCACTACCCAATCTAAATGAGG 3') and M5 (ReV 5' GTTTGTAACATGCCACAGACATC 3') (Lafon et al., 1998) and the second was done with the primers JLP1 (Fw 5' CTCATGTGGGAGGCTGTGACCT 3') and M5 (Fw 5' GTTTGTAACATGCCACAGACATC 3') (Agostini et al., 1996). PCR amplification was run in a reacting volume of 25 µL containing 12.5µL PCR Master Mix (QIAGEN Taq PCR Master Mix, Hilden Germany) 1µL (1µM) FW and 1µL (1µM) REV primers, 5µL previously isolated DNA and 5.5µL injection grade water. PCR was car-

ried out in a thermocycler Master Cycler Gradient (Eppendorf, Germany) following several steps: an initial denaturation at 95°C for 5 min, followed by 40 cycles at 95°C for 30 s, 60°C for 40 s, 72°C for 60 s, and terminal elongation at 72°C for 10 min.

Visualization of PCR products of appropriate length were performed by electrophoresis in 2% agarose gel stained with ethidium bromide.

#### Statistical analyses

The chi-square test was used for statistical analyses. P values <0.05 were considered statistically significant and P values < 0.01 were considered highly statistically significant.

## RESULTS

JCV DNA was detected in 19/65 (29.2%) and BKV DNA in 3/65 (4.6%) urine samples collected from 65 healthy volunteers or nonimmunocompromised patients. Forty-three (66,2%) urine samples of the immunocompetent donors were negative for both viruses and none of the samples were positive for both viruses (Table 1). Statistical analyses showed that there was a highly statistically significant difference in the detection of JCV DNA and BKV DNA in the patients included in this study ( $\chi^2=14.007$ ;  $p<0.01$ ). Eleven of 19 JCV DNA-positive urine samples were collected from males and 9/19 from females. Among 3/65 BKV DNA-positive urine samples, two were collected from a male and one from a female patient (Table 1). There was no statistically significant difference in the excretion rates of BKV or JCV between male and female donors ( $\chi^2=0.697$ ;  $p> 0.05$  for BKV;  $\chi^2=0.013$ ;  $p> 0.05$  for JCV).

## DISCUSSION

BKV and JCV are the most prevalent human polyomaviruses worldwide. After primary infections, these viruses persist in the kidneys as a latent infection. Reactivation of these viruses can occur in both immunosuppressed and nonimmunocompromised individuals. BKV and JCV reactivation in immunocompetent hosts does not cause any renal damage and the shedding of these viruses in urine is not connected with any clinical symptom. The behavior of these viruses in healthy host is still an enigma and to date little is known about viral reactivation and shedding over time.

In this study, we found that the overall frequency rate of JCV and BKV in the urine of immunocompetent individuals was 29.2% and 4.6% respectively. In other countries, the reported frequency of JCV viruria varied from 19% in Switzerland (Egli et al., 2009) to 21% in Ireland (Schaffer et al., 2006), 32% in Germany (Agostini et al., 2001), 38% in Portugal (Matos et al., 2010), 39-46% in Poland (Agostini et al., 2001; Kmiecik et al., 2008), 40-47% in Italy (Ferrante et al., 2001; Pagani et al., 2003; Rossi et al., 2007), 47-54% in Spain (Agostini et al., 2001; Polo et al., 2004), and approximately 5% of immunocompetent individuals have BKV viruria (Doerries, 2001). Therefore, the prevalence of JCV and BKV viruria found in the Serbian population is in accordance with results from other European countries.

Most of these studies did not show significant difference in the excretion rates of BKV or JCV between males and females (Kitamura et al., 1990; Ling et al., 2003; Pagani et al., 2003; Zhong et al., 2007; Kmiecik et al., 2008; Matos et al., 2010) as was observed in the present study.

In conclusion, our results confirm that BKV and JCV are not only found in the urine of patients with immunosuppression but can be found in the urine of immunocompetent individuals as well, and that JCV viruria is more prevalent than BKV viruria in healthy and nonimmunocompromised subjects. In addition, this study provides the first results of urinary excretion

of human polyomaviruses in the Serbian population and our findings are similar to those observed in other European countries. Further studies are required to determine the prevalence of human polyomaviruses among immunosuppressed patients and to determine the genotypes of BKV and JCV that are circulating among the Serbian population.

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