PREVALENCE OF JC AND BK POLYOMAVIRUS EXCRETION IN THE URINE OF HIV-INFECTED PATIENTS FROM SERBIA

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Abstract - BK virus (BKV) and JC virus (JCV) persist as latent infection in the kidneys. Reactivation of both viruses may be linked to immunodeficiency. The aims of this study were to determine the prevalence of BKV and JCV viruria and to evaluate the relationship between immunodeficiency and viruria in a cohort of HIV-infected patients. Urine samples from 93 HIV-infected patients were collected and tested for the presence of BKV and JCV DNA by PCR. The overall prevalence of polyomavirus DNA in urine was 74.2%. BKV DNA was detected in 30.1% urine samples and JCV DNA in 23.7% samples. Both BKV and JCV DNA were detected in 20.4% samples. There was no association between BKV/JCV urinary shedding and the degree of immunosuppression measured by CD4+ cell count. However, taking into account the severity of disease resulting from reactivation of BKV and JCV, patients with HIV/polyomavirus co-infection should be kept under frequent and regular supervision.

Key words: JC virus, BK virus, HIV-infection, immunosuppression, urinary excretion

INTRODUCTION

BK virus (BKV) and JC virus (JCV) are worldwide-distributed polyomaviruses in the human population. Primary infection with both viruses, which occurs mainly during early childhood, is usually asymptomatic or linked to mild respiratory disease (Goudsmit et al., 1981). After primary infection, both viruses persist as latent infection in the kidneys and upper part of the urinary tract (Chester et al., 1983).

Epidemiological studies have shown that seroprevalence is very high for both viruses, ranging from 70 to 90% (Gardner et al., 1973; Padgett and Walker, 1973). Seroconversion for BKV occurs in children aged 5 to 7, while JCV seroconversion usually occurs after the age of 10 (Knowels, 2006). The BKV antibody titer decreases throughout life, while antibodies against JCV remain stable and increase during life (Knowels et al., 2003; Jiang et al., 2009).

Asymptomatic shedding of BKV has been described in 5% of healthy adults, while 20 to 30% of seropositive immunocompetent adults sporadically shed JCV in the urine (Doerries, 2001). Higher incidence of reactivation is observed in immunocompromised patients (HIV-infected patients, cancer patients, transplant organ recipients, etc.) (Gardner et al., 1984; Dubois et al., 1996; Arthur et al., 1988). In recent years, several immunomodulatory drugs (monoclonal antibodies) used for the treatment of
autoimmune diseases, as a side effect lead to increased reactivation of both viruses, especially JCV (Carson et al., 2009; Major, 2010). In immunocompromised individuals, the reactivation of human polyomavirus can cause severe and life-threatening diseases. Reactivation of JCV results in lytic infection of oligodendrocytes in the brain and the development of progressive multifocal leukoencephalopathy (PML) (Jiang et al., 2009), while reactivation of BKV is associated with hemorrhagic cystitis, ureteral stenosis and polyomavirus induced nephropathy (Apperley et al., 1987; Gardner et al., 1971).

Knowing the severity of diseases associated with the reactivation of human polyomaviruses in immunocompromised individuals, the aims of this study were to determine the prevalence of BK and JC viruria and critically evaluate the relationship between immunodeficiency and viruria in a cohort of HIV-infected patients in Serbia.

MATERIALS AND METHODS

Urine samples and subjects

Urine samples were collected from 93 non-selected consecutive HIV-infected patients who were undergoing regular follow-up at the Outpatient Unit of the Clinic of Infectious and Tropical Diseases, Clinical Center of Serbia.

Single urine samples were collected from all individuals and stored at -70°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 No 29/VI-12.

DNA extraction and amplification of JCV VP1 and BKV VP1 regions

Ten milliliters of urine were centrifuged at 4 000g for 10 min. The sediment was re-suspended in 5 ml of sterile phosphate-buffered saline (PBS) and recentrifuged. The supernatant was discarded and viral DNA was then extracted by using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer’s instructions.

Seminested-PCR was performed for amplification of the 495-bp fragment within the VP1 coding region of the JCV genome. Primers P13 and M5 were applied for the first PCR round, and JLP1 and M5 for the second round (Table 1). PCR was carried 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 final extension at 72°C for 10 min.

The BKV VP1 region was amplified by seminested-PCR where BKV1 and BKV2 were used as outer primers, and BKV3 and BKV2 as inner primers (Table 1). Amplification of BKV VP1 was performed under the same conditions as described above with the only difference in the annealing temperature, which was at 60°C for 1 min.

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

Statistical analyses

The chi-squared test was used for statistical analysis. Analysis was performed in SPSS v.20 (SPSS Inc., Chicago, IL) software. P<0.05 was considered significant.

RESULTS

Ninety-three HIV-infected patients (70 men, 23 women) were enrolled in this study. The mean age was 44.24±10.63 years (range 23-70). The CD4+ T cell count was determined for 88/93 patients and more than half of the patients (58%) presented CD4+ cell counts <200 cells/mm³.

The overall prevalence of polyomavirus DNA urinary shedding was 69/93 (74.2%), while 25.8% urine samples were negative for both viruses. The overall prevalence of BKV and JCV was 50.5% and 44.1%, respectively. Only BKV DNA was detected in 28/93
(30.1%) urine samples and only JCV DNA was detected in 22/93 (23.7%) samples. Both BKV and JCV DNA were detected in 19/93 (20.4%) samples. There was no statistically significant difference in detection rates of BKV DNA and JCV DNA among the patients participating in this study ($\chi^2=0.517; p=0.472$).

JCV DNA was more frequently detected among males than females and the detection rate of JCV DNA was significantly higher in men than in women ($\chi^2=4.016; p=0.045$) (Table 2). On the other hand, the detection rate of BKV DNA was similar between genders and the difference in detection rate of BKV DNA between genders did not reach statistical significance ($\chi^2=0.033; p=0.856$) (Table 2).

The number of positive samples for BKV and JCV were analyzed when all patients were grouped by age. The prevalence of JCV and BKV in HIV-infected patients grouped by age is shown in Table 3. The data suggests that the prevalence of JCV and BKV does not significantly differ between different age groups ($P$ value NS).
There was no statistically significant difference in the proportion of positive samples between the age groups ($\chi^2=5.051, p =0.654$ for JCV; $\chi^2=6.822, p =0.448$ for BKV) (Table 3).

In order to evaluate the relationship between BKV/JCV viruria and immunodeficiency, HIV-infected patients were divided into three groups according to their CD4+ cell count: <200, 200-500, and >500 x 10^6 cells/L. The data showed no correlation between the frequency of BKV/JCV urinary excretion and the degree of immunosuppression (measured by CD4+ cell count) (Tables 4 and 5).

### Table 4. Prevalence of JCV viruria in HIV infected patients based on CD4+ cell count subdividing

<table>
<thead>
<tr>
<th>CD4+ (per mm3)</th>
<th>No. of JCV positive (%)</th>
<th>No. of JCV negative (%)</th>
<th>Total</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200</td>
<td>24 (27.3)</td>
<td>27 (30.7)</td>
<td>51 (58)</td>
<td>NS</td>
</tr>
<tr>
<td>201-500</td>
<td>13 (14.7)</td>
<td>19 (21.6)</td>
<td>32 (36.3)</td>
<td>NS</td>
</tr>
<tr>
<td>500+</td>
<td>2 (2.3)</td>
<td>3 (3.4)</td>
<td>5 (5.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>39 (44.3)</td>
<td>49 (55.7)</td>
<td>88 (100)</td>
<td></td>
</tr>
</tbody>
</table>

NS - non significant

### Table 5. Prevalence of BKV viruria in HIV-infected patients based on CD4+ cell count subdividing

<table>
<thead>
<tr>
<th>CD4+ (per mm3)</th>
<th>No. of BKV positive (%)</th>
<th>No. of BKV negative (%)</th>
<th>Total</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200</td>
<td>29 (33)</td>
<td>22 (25)</td>
<td>51 (58)</td>
<td>NS</td>
</tr>
<tr>
<td>201-500</td>
<td>13 (14.7)</td>
<td>19 (33)</td>
<td>32 (36.3)</td>
<td>NS</td>
</tr>
<tr>
<td>500+</td>
<td>2 (2.3)</td>
<td>3 (3.4)</td>
<td>5 (5.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>44 (50)</td>
<td>44 (50)</td>
<td>88 (100)</td>
<td></td>
</tr>
</tbody>
</table>

NS - non significant

In the present study, the prevalence of BKV in HIV-infected patients who were shedding only BKV was 30.1%. However, the overall prevalence of BKV was higher when patients with the simultaneous excretion of both viruses is considered, reaching 50.1%. This result is similar to those reported previously by Knowels et al. (1999) and Nali et al. (2012), although lower rates of less than 25% were detected by Sundsfjord et al. (1994). The reason for this may be due to the characteristics of the patients included in the studies, especially their immunological status (level of immunosuppression, CDC stage of disease etc.).

The overall prevalence of JCV DNA in this study was as high as 44.1%. This frequency is similar to the detection rate of JCV DNA reported by Matos et al. (2010), although higher than most so-far published data where JCV DNA was detected in the urine of 16 to 32% of HIV-infected patients (Sundsfjord et al., 1994; Knowles et al., 1999; Markowitz et al., 1993).
This study showed a higher prevalence of BK viruria than JC viruria in HIV-infected patients but no statistically significant difference was reached. The results of our previous study (Karalic et al., 2012) involving immunocompetent Serbian population showed that the prevalence of JCV urinary shedding is significantly higher than BKV shedding (29.2% v. 4.6%). Furthermore, when the results of both studies are compared there was no statistically significant difference in JCV excretion rate between immunocompetent individuals and immunocompromised HIV-infected patients, indicating that immunosuppression has little effect on the reactivation of JCV and that probably some other factors are involved in its reactivation. On the other hand, a significantly higher rate of BK viruria in HIV-infected patients suggests that immunosuppression is one of the most important factors for the reactivation of BKV.

No association between BKV/JCV urinary shedding and degree of immunosuppression measured by CD4+ cell count was found. This finding is similar to results from other studies (Behzad-Behbahani et al., 2004; Matos et al., 2010; Sundsfjord et al., 1994). On the other hand, studies done by Markowitz et al. (1993), Knowles et al. (1999) and Jin et al. (1995) found a correlation between BKV shedding and the degree of immunosuppression; the lower the CD4+ cell count the higher excretion rate of BKV was, but no association of JCV urinary excretion with immunodeficiency was found.

The majority of previously published studies (Kitamura et al., 1990; Kmieciak et al., 2008; Matos et al., 2010; Nali et al., 2012) found no association between gender and excretion rate of BKV/JCV. However, the results of this study and the study of Rossi et al. (2007) showed that men were more likely to excrete JCV in urine than women.

In the immunocompetent population, JCV urinary excretion rate is age-related; in the older population the excretion rate was higher (Kitamura et al., 1990; Polo et al., 2004), but no association between urinary shedding of BKV with age was determined (Zhong et al., 2007; Kitamura et al., 1990). On the other hand, this study confirmed findings from other studies (Knowles et al., 1999; Nali et al., 2012) where no statistically significant difference was observed between excretion rates of BKV/JCV and the age of patients in immunocompromised HIV-infected patients.

In conclusion, this study showed a high prevalence of BKV and JCV DNA in the urine of HIV-infected patients. BK viruria was more prevalent than JC viruria. This study confirmed that immunosuppression is the most important factor for the high prevalence of BKV reactivation, while some additional factors are needed for the reactivation of JCV. The severity of disease resulting from reactivation of BKV and JCV in patients with HIV/polyomavirus co-infection should be kept under more frequent and regular supervision.

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REFERENCES


