

## Human polyomaviruses JC and BK in the urine of Brazilian children and adolescents vertically infected by HIV

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*The aim of this study was to characterize the urinary excretion of the BK (BKV) and JC (JCV) human polyomaviruses in a cohort of human immunodeficiency virus (HIV)-infected children and adolescents. One hundred and fifty-six patients were enrolled: Group I included 116 HIV-infected children and adolescents [median age = 11.4 years (y); range 1-22 y]; Group II included 40 non-HIV-infected healthy controls (median age = 11.37 y; range 7-16 y). Single urine samples from both groups were screened for the presence of JCV and BKV DNA by polymerase chain reaction at enrolment. The overall rate of JCV and BKV urinary excretion was found to be 24.4% and 40.4%, respectively (n = 156). Group I had urinary excretion of JCV and BKV in 27.6% and 54.3% of subjects, respectively. In contrast, Group II showed positive results for JCV in 17.5% of subjects and for BKV in 12.5% of subjects (p Pearson JCV = 0.20; p Pearson BKV < 0.0001). In Group I, there was no association between JCV/BKV shedding and age, gender or CD4 values. Patients with an HIV viral load < 50 copies/mL had a lower excretion of BKV (p < 0.001) and a trend of lower JCV excretion (p = 0.07). One patient in Group I (1/116, 0.9%) showed clinical and radiological features consistent with progressive multifocal leukoencephalopathy, suggesting that children with HIV/polyomavirus coinfection should be kept under surveillance.*

Key words: HIV-1 - child - adolescent - JC virus - BK virus - urine

Humans are the natural hosts for JC virus (JCV) and BK virus (BKV), two viruses classified within the *Poliomaviridae* family. The viruses are usually non-pathogenic for non-immunocompromised individuals, but reactivation in immunocompromised patients with cellular immunodeficiency has been associated with serious complications. Transmission is thought to occur through the respiratory route and perhaps by the faecal-oral route (Weimberg & Mian 2010).

The most common site of BKV latency in humans is the urogenital tract and viral sequences can be detected in up to 50% of human kidneys. Peripheral blood mononuclear cells (PBMC) are a second important site of BKV latency. Compared to healthy subjects, there is an increased incidence of viruria in human immunodeficiency virus (HIV)-infected individuals, ranging from 20-44% in most studies (Markowitz et al. 1993, Sundsfjord et al. 1994, De-gener et al. 1997, Behzad-Behbahani et al. 2004).

JCV is neurotropic and reactivation of its latent infection causes progressive multifocal leukoencephalopathy (PML), a fatal demyelinating neurological disease, in immunocompromised individuals. Reactivation of hu-

man polyomavirus may also occur in otherwise healthy people, leading to asymptomatic viruria (Major & Ault 1995). Many questions concerning the pathogenesis and natural history of BKV and JCV infection remain unanswered, especially in children and adolescents exposed to HIV-infection. The objectives of this observational study were: (i) characterise JCV and BKV viruria in a group of HIV-1-infected children and adolescents and in a group of healthy controls and (ii) describe clinical and laboratory parameters of the HIV-infected subjects that were positive for BKV and/or JCV viruria during the study period.

### PATIENTS, MATERIALS AND METHODS

*Study subjects and samples* - One hundred and fifty-six subjects were enrolled in this study from March 2007-April 2008. The subjects were divided into two groups. The first group (Group I) included 116 HIV-infected children and adolescents followed at the Paediatric AIDS Outpatient Clinic of the Federal University of São Paulo (UNIFESP), Brazil. The second group (Group II) included 40 non-HIV-infected healthy controls selected by age in a public elementary school after the parents provided a signature of informed consent. The stage of immunodeficiency in the HIV infected individuals was defined according to the CDC Classification (1994). Clinical characteristics of Group I are shown in Table I.

The protocol was approved by the Ethical Committee of the UNIFESP and all parents gave written informed consent prior to their child's enrolment in the study. After obtaining informed consent, a urine sample from

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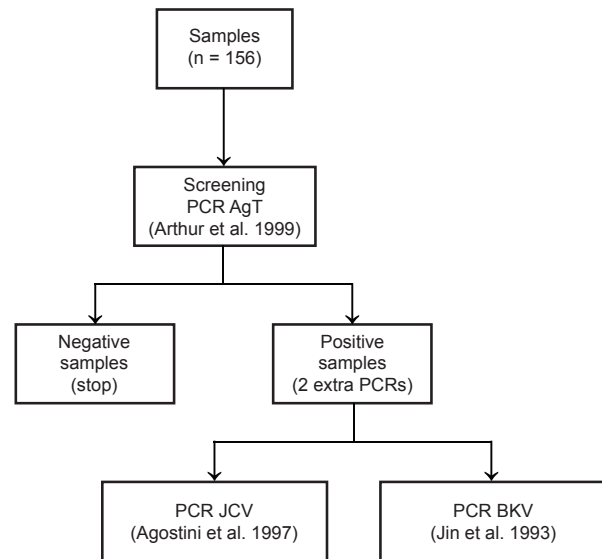
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each patient was screened using a standard polymerase chain reaction (PCR) technique for the presence of JCV and BKV DNA at enrolment. Samples were sent to the Laboratory of Virology, Institute of Tropical Medicine, São Paulo, Brazil and stored at -70°C until processing.

*Detection of JCV and BKV DNA in urine samples* - JCV and BKV DNA was extracted from 200 µL of urine samples by resin columns (QIAmp® DNA Blood Mini Kit, Qiagen, Germany) according to the manufacturer's instructions. The algorithm explaining the strategy used for the identification of positive samples for both human polyomavirus BK and JC is shown in Figure.

DNA amplification of a region common to both JCV and BKV was used for screening. Amplification was performed using standard PCR technique in a Perkin Elmer 2400 or PTC 100 thermocycler. The set of primers PEP-1 and PEP-2, which were described by Arthur et al. (1999) (5'-AGTCTTTAGGGTCTTCTACC-3', 5'-GGTGCCAACCTATGGAACAG-3') and amplify the T-antigen genes (173 bp) of both JCV and BKV, was used. The PCR mixture (50 µL) contained 10 µL of extracted DNA, deoxynucleotide triphosphates at a concentration of 200 µM each, 0.5 µM of primers, 1 × reaction buffer (10 mM Tris, 50 mM KCl, pH 8.3), 1.5 mM MgCl<sub>2</sub> and Taq (2.5 U/reaction). The target DNA was amplified using 40 thermal cycles (92°C for 1.5 min, 55°C for 1.5 min, 72°C for 2 min, with a final elongation step of 7 min). Samples that were positive for T antigen were submitted to two extra PCRs to discriminate between the JCV and BKV. JCV DNA was detected by amplifying a 215 bp



Algorithm showing the strategy used for the identification of positive samples for both human polyomavirus BK and JC. BKV: BK virus; JCV: JC virus; PCR: polymerase chain reaction.

fragment of the *VPI* gene in a one step PCR reaction (nucleotides 1710-1734 and 1924-1902) (Agostini et al. 1997). The amplification was performed according to Shaffer et al. (2006). BKV DNA was detected by amplifying a 354 nucleotide region within the *VPI* gene

TABLE I  
Characteristics of the study population

	Group I (n = 116)	Group II (n = 40)
Age in years (mean; range)	11.4; 1-20	12; 7-16
Gender [n (%)]		
Male	63 (54)	30 (75)
Female	53 (46)	10 (25)
HIV immunologic category [n (%)]		
1	23 (19.8)	-
2	45 (38.8)	-
3	48 (41.4)	-
HIV clinical category [n (%)]		
N/A	26 (22.4)	-
B	52 (44.8)	-
C	38 (32.8)	-
HIV viral load < 50 (copies/mL) at inclusion [n (%)]	35 (30.2)	-
HIV viral load < 400 (copies/mL) at inclusion [n (%)]	60 (51.7)	-
HIV viral load after 24 months of follow-up [n (%)]		
< 50 (copies/mL)	44 (38.3)	-
≤ 10,000	50 (43.5)	-
> 10,000	21 (18.3)	-
CD4 nadir (cells/mm <sup>3</sup> ) (mean; range)	385; 4-1288	-
CD4 at inclusion (cells/mm <sup>3</sup> ) (mean; range)	671; 18-2023	-

HIV: human immunodeficiency virus.

(nt 1663-2016, DUN numbering) using the oligonucleotides BKV-1 (50-GAAGTTCTAGAAGTTAAACTGGG-30) and BKV-2 (50-GTGGAAATTACTGCCTTGAATAGG-30) (Jin et al. 1993).

*Statistical analyses* - Univariate analysis was performed to evaluate the variables significantly associated with JCV and BKV shedding in HIV-infected children. The following variables were included: age, clinical category, immunological category, viral load < 400 copies/mL and viral load < 50 copies /mL. Comparisons between groups were performed using the Pearson chi-square test ( $\chi^2$ ) for categorical variables. The level of significance was set at  $p < 0.05$  (SPSS version 15.0).

**RESULTS**

We collected 116 samples from Group I (n = 116) and 40 samples from Group II (n = 40). The overall rates of JCV and BKV urinary excretion were found to be 25% and 40.4%, respectively, including all persons enrolled in this study (n = 156).

Group I exhibited urinary excretion of JCV and BKV in 27.6% and 54.3% of subjects, respectively. In contrast, Group II showed positive urinary results for 17.5% of subjects for JCV ( $p = 0.20$ ) and for 12.5% of subjects for BKV ( $p < 0.0001$ ). Twelve of the 116 patients included in Group I (10.4%) had JCV/BKV coinfection, which was not seen in the subjects from Group II. The demographic characteristics and the occurrence of polyomavirus excretion in the urine of the study population are shown in Table II.

Although JCV and BKV DNA was more frequently detected in HIV-infected subjects than in non-infected subjects, univariate analysis did not show any statisti-

TABLE II  
Prevalence of BK virus (BKV) and JC virus (JCV) DNA in urine samples from human immunodeficiency virus-infected and non-infected patients

	Group I (n = 116)	Group II (n = 40)	
Urine samples	n (%)	n (%)	p values
JCV positive	32 (27.6)	7 (17.5)	0.20
BKV positive	63 (54.3)	5 (12.5)	< 0.001
Coinfection JCV/BKV	12 (10.4)	0 (0)	-

cally significant correlation between the frequency of JCV or BKV DNA detection and an HIV viral load of < 400 copies/mL or the stage of immunodeficiency of the HIV-infected group for either immunological (1 vs. 2 or 3) or clinical (N or A vs. B or C) categories (Table III).

Patients with HIV viral load below 50 copies/mL were unlikely to be shedding BKV ( $p < 0.001$ ). Statistical significance was not reached for JCV ( $p = 0.07$ ).

*Clinical aspects* - All control subjects and the majority of the HIV-infected patients were asymptomatic at the time of sampling. During the study period, the patients showing BKV or JCV viruria presented the following signs and symptoms: hyporeflexia (n = 1/116; 0.86%), seizures (n = 1/116; 0.86%), HIV encephalopathy (n = 1/116; 0.86%) and learning deficit (n = 1/116; 0.86%). One patient from Group I presented severe neurologic signs and symptoms during the study period. This pa-

TABLE III  
Demographic, clinical and laboratory variables according to JC virus (JCV) and BK virus (BKV) serological status in human immunodeficiency virus (HIV)-infected patients

Variable	JCV+	JCV-	p	BKV+	BKV-	p
Age (years)	n (%)	n (%)	0.55	n (%)	n (%)	0.8
1-4.99	2 (1.7)	5 (4.3)	-	3 (2.6)	4 (3.4)	-
5-9.99	7 (6)	27 (23.3)	-	20 (17.2)	14 (12.1)	-
≥ 10	23 (19.8)	52 (44.8)	-	35(30.2)	40 (34.5)	-
Clinical category						
N or A	6	18	-	15	9	-
B or C	26	66	-	43	49	-
Total	32	84	0.75	58	58	0.36
Immunological category						
1	6	16	-	9	13	-
2 or 3	26	68	-	49	45	-
Total	32	84	0.97	58	58	0.35
HIV viral load (copies/mL)						
< 50	14	22	0.07	11 (9.5)	25 (21.6)	0.007
< 400	17	43	0.85	30	30	-
400 < 10,000	8	15	-	10	13	-
10,000-100,000	7	21	-	15	13	-
> 100,000	0	5	0.45	3	2	0.81

tient showed clinical and radiological features compatible with PML, such as behaviour disorder, aggression, balance disturbance with walking difficulties, tremors of the extremities and characteristic magnetic resonance imaging (MRI) changes. This patient had positive results for BKV in both their urine and cerebrospinal fluid (CSF) samples. There was slow but progressive improvement of the neurological symptoms and recovery of the neurologic deficit after a mean time of six months. Despite neurological improvement, the patient presented nephrotic range proteinuria and chronic renal failure, dying 36 months after inclusion in the study.

## DISCUSSION

In the present study, 30% of healthy individuals (12/40) had detectable JCV or BKV DNA in their urine. This excretion rate is similar to that published previously by other authors. Behzad-Behbahani et al. (2004) found that excretion rates of JC or BK viruria varied from 33-60% in non-HIV-infected individuals. Matos et al. (2010) observed urinary JCV DNA in 33% of healthy individuals, a detection rate significantly lower than that observed for patients infected with HIV, which was 51%.

Increased incidence of polyomavirus viruria is observed in HIV-infected individuals (Lafon et al. 1998, Weimberg & Mian 2010). In our study, the frequency of BK viruria was significantly higher in HIV-infected patients in comparison to healthy control subjects (54.3% vs. 12.5%,  $p < 0.0001$ ), which may be explained by the T cell immunodeficiency in HIV-infected patients. However, a significant difference was not observed in JC viruria between HIV-infected and control groups (27.6% vs. 17.5%, respectively,  $p = 0.20$ ). Similar results have been reported by other studies (Markowitz et al. 1993, Behzad-Behbahani et al. 2004).

In a previous study in HIV-positive individuals, JCV and BKV sequences were found in 31.6% and 8.1%, respectively, of urine samples, whereas the positive test rate was 22.8% for JCV and as high as 51.1% for BKV in PBMCs (Sundsfjord et al. 1994).

BKV/JCV co-infection was frequent in Group I (10.4%), but was not detected in Group II. Other studies also observed that simultaneous shedding of both BKV and JCV DNA in urine samples was significantly higher in HIV-infected patients (25.5%) than in HIV-negative partners (10%) or patients attending a genitourinary medicine clinic (1.6%) (Behzad-Behbahani et al. 2004).

Patient age, clinical or immunological category and HIV viral load did not show an association with urinary JCV or BKV shedding. The only variable significantly associated with a decreased frequency of BKV shedding in urine was undetectable HIV viral load. Paradoxically, the same was not observed for JCV. These data suggest that, besides T cell immunodeficiency, other factors that are not well understood may cause JCV replication.

Several studies have shown that in individuals with immunodeficiency, reactivation of JCV may occur in oligodendrocytes and astrocytes, leading to PML (Antinori et al. 2001, Drake et al. 2007). The diagnosis is mainly based on clinical data, images of demyelination from MRI and brain biopsy data. The involvement of

HIV-1 secreted products has been proposed as a possible mechanism for stimulating JCV replication and facilitating the spread of this virus to the central nervous system (CNS) (Chowdhury et al. 1992, 1993).

In the present study, most of the patients were asymptomatic during polyomavirus shedding. Interestingly, one patient who presented with neurological manifestations had polyomavirus (BKV) in their urine and CSF and was diagnosed with PML. Although BKV is considered a nephrotropic virus and JCV a CNS-tropic virus, a few cases of BK CNS disease have been reported (Weimberg & Mian 2010). Although BKV infections in the CNS have been reported less frequently than those related to JCV (Fink et al. 2006, Vidal et al. 2007), there is evidence for BKV occurrence in the CNS of immunocompromised hosts. Therefore, in patients with neurological alterations and preceding or concurrent signs of renal involvement with BK viruria, the possibility of BK CNS disease should be investigated (Bratt et al. 1999).

Other patients in this study had signs and symptoms that are frequently found in HIV-infected patients and could be unrelated to the HIV/JCV or HIV/BKV coinfection. The high frequency of urinary polyomavirus excretion reflects the possibility of persistent or intermittent replication of the viruses. Also, it might be explained by the activation of viral replication caused by the interaction between HIV and polyomavirus, as suggested by the presence of a higher excretion of both BKV and JCV in patients with an HIV viral load  $> 50$  copies/mL, although only BKV excretion was statistically significant ( $p = 0.007$ ).

The time between reactivation in immunocompromised patients and the emergence of PML is not known. The presence of positive responses to antiretroviral treatment, as evidenced by HIV viral load  $< 50$  copies/mL in 38.3% of patients and subsequent maintenance of an adequate immune status could be decisive for the appearance or absence of a demyelinating status.

In this study, although 66.9% of patients had already shown moderate or severe immunosuppression in the past, there was an improvement in immune status in the subjects, as evidenced by an increase in median CD4 counts when compared to CD4 nadir counts. As many patients are still presenting HIV replication (61.8%), there is a concern about deterioration of the immune population in a short or moderate timeframe, with the possibility of reactivation of JCV and BKV viruses and subsequent clinical deterioration. The long-term consequences of co-infection with HIV and polyomavirus should be kept under constant surveillance in the study group.

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