JC polyomavirus infection in candidates for kidney transplantation living in the Brazilian Amazon Region

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This study evaluated the relative occurrences of BK virus (BKV) and JC virus (JCV) infections in patients with chronic kidney disease (CKD). Urine samples were analysed from CKD patients and from 99 patients without CKD as a control. A total of 100 urine samples were analysed from the experimental (CKD patients) group and 99 from the control group. Following DNA extraction, polymerase chain reaction (PCR) was used to amplify a 173 bp region of the gene encoding the T antigen of the BKV and JCV. JCV and BKV infections were differentiated based on the enzymatic digestion of the amplified products using BamH*I endonuclease. The results indicated that none of the patients in either group was infected with the BKV, whereas 11.1% (11/99) of the control group subjects and 4% (4/100) of the kidney patients were infected with the JCV. High levels of urea in the excreted urine, low urinary cellularity, reduced bladder washout and a delay in analysing the samples may have contributed to the low prevalence of infection. The results indicate that there is a need to increase the sensitivity of assays used to detect viruses in patients with CDK, especially given that polyomavirus infections, especially BKV, can lead to a loss of kidney function following transplantation.*

Key words: polyomavirus infection - JCV - BKV - chronic renal disease - kidney transplant

Two human polyomaviruses (BK and JC) were discovered more than 40 years ago (Gardner et al. 1971, Padgett et al. 1971). The JC virus (JCV) is the causative agent of progressive multifocal leukoencephalopathy (Padgett et al. 1971), which primarily affects immunosuppressed acquired immune deficiency syndrome (AIDS) patients. The BK virus (BKV) is associated with disorders such as haemorrhagic cystitis, urethral stenosis and other urinary tract diseases most commonly found in patients awaiting transplants or undergoing immunosuppressive therapy (Drachenberg et al. 2007).

The two viruses are widespread in the human population, with prevalence of 63% for BKV and 80% for JCV (Padgett et al. 1971, White & Khalili 2004). Primary infection with JCV usually occurs during childhood and is asymptomatic (Padgett & Walker 1973). At the site of entry, which is normally the respiratory tract, these viruses replicate and traffic to the tissues of the kidneys (Heritage et al. 1981, McCance 1983) and the central nervous system, where they sustain a long-term infection (Elsner & Dörries 1992, Quinlivan et al. 1992). A recurrence of the infection may be induced by significant immunosuppression, as in the case of kidney (Hogan et al. 1980, Kahan et al. 1980, Gardner et al. 1984) and bone marrow transplants (O'Reilly et al. 1981, Arthur et al. 1988) and other factors such as immunodeficiency diseases, diabetes, other chronic diseases, immunosuppressive chemotherapy, pregnancy (Coleman et al. 1980) and old age (Tajima et al. 1990).

Primary infection with BKV possibly occurs through the respiratory tract during early childhood, with the virus remaining dormant in the urinary tract and other organs (Goudsmit et al. 1982). This primary infection is usually asymptomatic, but it may induce a fever and nonspecific respiratory symptoms (Reploeg et al. 2001). This virus can also be transmitted through organ transplantation (Fioriti et al. 2005).

Reactivation of the BKV may occur spontaneously in immunocompetent subjects and is relatively frequent in individuals with impaired cellular immunity, such as pregnant women, cancer patients receiving chemotherapy, AIDS patients and transplant recipients. In these patients, the virus can cause haemorrhagic cystitis, nephritis, urethral stenosis and a loss of function of the transplanted kidney (Coleman et al. 1978, Gardner et al. 1984, Chan et al. 1994, Weiskittel 2002).

The presence of BKV has been associated with rejection of the transplanted kidney, whereas JCV has been associated with nonspecific symptoms in the transplanted kidney, lungs and pancreas (Weiskittel 2002).

Reactivation of BKV is now responsible for 10-60% of the cases of the loss of function of the grafted organ in kidney transplants (Hurault de Ligny et al. 2003). This polyomavirus causes tubulointerstitial nephritis in the kidney tissue, which is known as polyomavirus-associated nephropathy (Hirsch et al. 2006).

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The detection of polyomaviruses in kidney transplant candidates (patients with chronic renal disease) should be a standard component of pre-transplant research, especially given the potential role these viruses play in graft rejection (Pires et al. 2011). The differential diagnosis of these viruses is also essential, given that they are histologically similar, but require distinct courses of treatment (Elli et al. 2002).

This study aimed to evaluate the relative prevalence of JCV and BKV infections in kidney transplant candidates with chronic renal disease from the Brazilian state of Acre (AC) and the possible association between clinical or laboratory factors of disease and socio-demographic variables.

SUBJECTS, MATERIALS AND METHODS

Sample groups - Urine samples were collected from 199 volunteer subjects between April-July 2008. All subjects were over 18 years old and resided in Rio Branco, the capital of AC.

The experimental group consisted of 100 patients diagnosed with chronic kidney disease (CKD). All subjects were undergoing haemodialysis treatment at the Clinical Hospital of Acre (HCA).

The control group consisted of 99 patients from the HCA without any symptoms of acute or CKD or any other urinary tract disorder. All samples were sent to the Virus Laboratory of the Biological Sciences Institute of the Federal University of Pará (UFPA).

Ethics - All subjects were contacted prior to the study and provided with information on its aims. Those subjects who agreed to participate in the study by providing a urine sample were required to sign an informed consent form. The project was submitted to the Research Ethical Commission of the UFPA Medical Sciences Institute and was authorised under protocol 090/06. The study followed the guidelines and regulatory standards of the Brazilian National Health Council (Resolution 196/96) for research involving human subjects.

Sample collection - Urine samples (mean volume 15.2 ± 5.3 mL, range 5-50 mL) were collected in sterile flasks. At the UFPA Virus Laboratory, the samples were centrifuged at 3,640 *g* for 15 min until the urine residue was completely separated. The sediment was then washed three times with a sterile saline solution (0.9%) and the cell pellet was frozen at -20ºC until the DNA could be extracted, as described by Pires et al. (2011).

DNA extraction - DNA was extracted from the cell pellets following the protocol for viral nucleic acid extraction using a Link Pure Viral RNA/DNA kit (Invitrogen, CA, USA).

Molecular analysis - The presence of JCV and/or BKV was evaluated by amplifying a 173 bp fragment of the gene encoding the T antigen of both viruses, which was amplified by polymerase chain reaction (PCR) as described by Pires et al. (2011). DNA samples from previously confirmed JCV and BKV infected subjects, stored at -20ºC, were used as positive controls in all PCR assays.

JCV or BKV infections were confirmed by enzymatic digestion of the amplified product using the restriction endonuclease *BamH*I (2 U/μg of DNA) (Invitrogen, CA, USA), which cleaves the amplified product from JCV into 120 bp and 53 bp fragments, but does not cleave the amplified product from BKV. The products of this digestion were visualised using 3% agarose gel electrophoresis as previously described (Pires et al. 2011).

To test the quality of the extracted DNA and for the presence of polymerase inhibitors, all samples were used to amplify a 349 bp segment of the human gene Mbl2 as an internal control following a previously described protocol (Vallinoto et al. 2009).

Statistical analysis - Descriptive statistics, including absolute (n) and relative (%) frequencies or mean, standard deviation, median and range, were calculated separately for the two study groups. The significances of between-group differences in discrete socio-demographic variables (sex ratio, education level and place of birth) and the possible association between JVC infection and clinical variables were assessed using the chi-square or likelihood ratio tests, where applicable. For continuous variables, the Kolmogorov-Smirnov test was first applied to determine the normal distribution of the data and a *t* test was then used to compare mean ages and urea and creatinine levels between groups. A paired *t* test was used to compare pre and post-dialysis urea levels in the kidney patients. A significance level of 0.05 ($\alpha = 5\%$) was adopted and p values lower than this were considered significant. All statistical analyses were run on SPSS software for Windows, version 15.0 (Chicago, USA).

RESULTS

Social and demographic variables - There were no statistically significant differences between the experimental and control groups (Table I) for the sex ratio ($p = 0.523$), mean age ($p = 0.648$), age distribution ($p = 0.634$), education levels ($p = 0.241$) or birthplace ($p = 0.117$).

Clinical and laboratory analyses - The creatinine and urea serum levels of the subjects were recorded (Table II); in the case of urea, pre and post-dialysis levels were also recorded for the kidney patients (Table III). Mean urea serum levels were significantly higher ($p < 0.001$) in the experimental group (kidney patients) compared to the control group, as were the mean creatinine levels ($p < 0.001$). In the kidney patients, as expected, urea concentrations declined significantly following dialysis in all patients ($p < 0.001$), with a mean reduction of 86.4 ± 39.1 mg/dL, range = 17-191 mg/dL (Table III). Overall, 77 (77%) of the patients had post-dialysis levels of urea above 35 mg/dL (the upper limit of the normal range), whereas the values for the remaining 23 patients (23%) were within normal limits.

Prevalence of polyomaviruses - The JCV was identified in 11.1% (11/99) of the subjects in the control group and 4% (4/100) of those in the CKD patient group (Table IV). Five DNA samples from CKD patients were unable to amplify the 349 bp fragment of the human gene Mbl2. The BKV was not identified in any of the urine samples. There were no statistically significant differences between JCV infection and sex ($p = 0.773$), age ($p = 0.567$), race ($p = 0.078$) or place of birth, $p = 0.516$ (Table IV).

TABLE I

Socio-demographic characteristics of the two study groups

Variable	Subjects n (%) CKD patients (n = 100)	Control group (n = 99)	Total sample (n = 199)	p[a]
Education level				
Illiterate	19 (19)	26 (26.3)	45 (22.6)	0.241
Literate	33 (33)	28 (28.3)	61 (30.7)	
Elementary school	34 (34)	23 (23.2)	57 (28.6)	
High school	9 (9)	13 (13.1)	22 (11.1)	
University	5 (5)	9 (9.1)	14 (7)	
Place of birth				
Acre	77 (77)	63 (63.6)	140 (70.4)	0.117
Other Brazilian state	21 (21)	33 (33.3)	54 (27.1)	
Other country	2 (2)	3 (3)	5 (2.5)	

a: p values for the comparison between experimental and control groups; CKD: chronic kidney disease.

DISCUSSION

The polyomavirus infection rates recorded in this study were considerably lower than those reported in other Brazilian populations (Cayres-Vallinoto 2008, Pires et al. 2011). Cayres-Vallinoto (2008) reported a JCV infection rate of 33% in the city of Belém, state of Pará, whereas Pires et al. (2011) reported prevalence of 3.9% in patients diagnosed with CKD and 22.4% in asymptomatic subjects. In Europe, Rodrigues et al. (2007) analysed the excretion of polyomaviruses in the urine of Portuguese subjects and reported a prevalence of 25.7%. Additionally, polyomavirus infection rates may vary considerably between different sectors of the country's population. For example, Suzuki et al. (2002) reported an infection rate of 18% for Americans of European descent, but a rate of 53% for those of Japanese descent. The low rates of polyomavirus infection reported here may represent an epidemiological feature of the virus in AC, which may be related to the population density and environmental conditions, which are of paramount importance for JCV and BKV transmission (Bofill-Mas & Girones 2001, Bofill-Mas et al. 2003). Thus, it is crucial to investigate the infection rates in different regions of Brazil and in distinct ethnic groups to establish the real prevalence of these viruses.

In this study, JCV infection was rare and BKV infection was absent from the samples analysed, which may suggest possible problems with the PCR analysis resulting from either inadequate sample collection and processing or other factors arising during analysis. In particular, urine samples destined for PCR should not be stored for long periods prior to processing because there is a tendency for the sample to alkalinise, which may result in a false negative result (Khan et al. 1991). In this study, the time between the sample collection and the urine sample analyses (mean of 2 days) may have led to a change in the pH of the material, which could have inhibited the PCR, resulting in reduced viral detection.

TABLE II

Serum levels of urea and creatinine recorded in the samples collected from the two study groups

Substance	Concentration (mg/dL) CKD patients (n = 100)	Control group (n = 99)	p
Urea			
Mean (SD)	140.9 (45.9)	24.2 (6.4)	< 0.001
Median	134.0	23.0	
Minimum-maximum	43-236	12-35	
Creatinine			
Mean (SD)	11.04 (3.81)	0.96 (0.24)	< 0.001
Median	11.35	0.90	
Minimum-maximum	2.6-19.0	0.4-1.5	

CKD: chronic kidney disease; SD: standard deviation.

Two additional factors could also have been important in the case of the kidney disease patients: the high concentrations of urea in the pre-dialysis samples and the mucoid secretions produced by the urinary tract epithelium, which are among the substances known to inhibit PCR (Al-Soud & Rådström 1998). Thus, the differences in JCV prevalence between the study groups may have been at least partly due to the significant difference in urea levels. Additionally, Behzadbehbahani et al. (1997) observed that high levels of urea inhibited the amplification of segments of the BKV genome.

Similarly, subjects with bladder dysfunction due to low urine volume and low frequency of urination, which is typical in haemodialysis patients, tend to accumulate

TABLE III

Serum urea levels in the pre and post-dialysis samples obtained from the chronic kidney disease patients

Parameter (n = 100)	Concentration of urea (mg/dL)		Reduction (pre-post)	p
	Pre-dialysis	Post-dialysis		
Mean (SD)	140.9 (45.9)	54.6 (22.1)	86.4 (39.1)	< 0.001
Median	134.0	54.50	78.5	
Minimum-maximum	43-236	13-109	17-191	

SD: standard deviation.

large amounts of mucoid secretions in the bladder (Han et al. 2002). Once again, this may have been a factor that contributed to the PCR inhibition in this study.

Other factors (polymerase inhibitors) that may have interfered with the results, especially those of the experimental group, include changes in pH, chelation of magnesium ions and the abnormal quantities of haemoglobin and glycoproteins typically found in the urine of patients with CKD (Mahbudani & Bej 1974, Greenfield & White 1993, Bej & Mahbubani 1994, Panaccio & Lew 1994). Patients with chronic renal failure requiring haemodialysis (end-stage renal function-creatinine clearance < 20-15 mL/min/m²) also develop significant morphological changes in the renal parenchyma characterised histologically by glomerular hypertrophy, formation of intraglomerular thrombus, excessive proliferation of glomerular cells and mesangial matrix, glomerular deposition of lipids, stretching of the mesangial and endothelial cells, injury to the podocyte and subendothelial deposition of protein. These histological changes are characterised morphologically by the atrophy of the renal parenchyma with decreased volume and organ function (Romão 2004). Thus, the low cellularity of the urine (caused by renal atrophy) may have also contributed to reduced polyomavirus detection in the experimental group. These hypotheses involving polymerase inhibitors are being raised here because we were unable to amplify the 349 bp fragment of the human gene Mbl2 from a few of the patient samples.

As the polyomavirus replicates in the renal parenchyma, it seems likely that fewer viruses will be shed into the urine of individuals with reduced renal parenchyma activity. Once again, this may have contributed to the reduced prevalence of polyomavirus in the kidney patients reported here.

Finally, the results obtained here are of paramount importance because they highlight the clear need to improve the sensitivity of the methods used to detect polyomavirus in the urine of patients with chronic renal failure (kidney transplant candidates), especially given the potentially harmful effects of these viruses, which may cause a loss of function of the transplanted organ. As we have proposed previously (Pires et al. 2011), the use of a real-time PCR assay could be an alternative method for improving the sensitivity of detecting viral DNA even when there are low viral titres.

TABLE IV

Prevalence of JV virus in the study population by demographic variable

Variable	Subjects n (%)		p
	Negative	Positive	
Sex (n)			
Male (99)	91 (91.9)	8 (8.1)	0.773
Female (100)	93 (93)	7 (7)	
Age class [years (n)]			
< 20 (4)	4 (100)	0 (0)	0.567
20-29 (8)	7 (87.5)	1 (12.5)	
30-39 (24)	21 (87.5)	3 (12.5)	
40-49 (41)	37 (90.2)	4 (9.8)	
50-59 (58)	53 (91.4)	5 (8.6)	
60-69 (55)	53 (96.4)	2 (3.6)	
70-79 (9)	9 (100)	0 (0)	
Skin colour (n)			
White (60)	54 (90)	6 (10)	0.078
Black (39)	35 (89.7)	4 (10.3)	
Yellow (30)	26 (86.7)	4 (13.3)	
Indian brown (24)	24 (100)	0 (0)	
Negroid (46)	45 (97.8)	1 (2.2)	
Place of birth (n)			
Acre (140)	128 (91.4)	12 (8.6)	0.516
Other Brazilian state (54)	51 (94.4)	3 (5.6)	
Other country (5)	5 (100)	0 (0)	

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