

# First Detection of Human Polyomaviruses in HIV Patients with Suspected Neurological Disease in Montería, Colombia

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## Abstract

**Objective:** The objective is to establish the presence of JC and/or BK polyomavirus in HIV patients with symptoms of encephalitis and/or meningitis. **Methodology:** From September 2009 to December 2011, a prospective study was conducted. 34 HIV patients with symptoms consistent with encephalitis and/or meningitis were included. The work was conducted in 3 hospitals in the city of Monteria. Viral DNA extraction was performed on samples of cerebrospinal fluid (CSF) using a commercial kit (Quiagen, USA). The detection of BKV and JCV was performed by multiplex real-time PCR (LightMix®, Roche Diagnostics, Germany) with primers specific for the short t antigen gene fragment, labeled probes and one internal control. **Results:** In 9 (26%) of 34 patients included in the study, JCV virus was detected; only 1 (3%) patient had coinfection with JCV/BKV. The mortality rate was 3%. The cytochemical examination of CSF in positive patients presented average values: 40.7 mg/dL glucose, 171.66 mg/dL protein, 19.8 mm<sup>3</sup> leukocytes, and 109.8 mm<sup>3</sup> erythrocytes. **Conclusion:** The findings demonstrate that JCV and BKV have a significant occurrence in HIV patients with CSF infections in Monteria.

## Keywords

Progressive Multifocal Leukoencephalopathy, Meningitis, Encephalopathy, AIDS

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## 1. Introduction

Human polyomaviruses belong to the family Papovaviridae. BK virus (BKV) and JCV virus are the principal

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members of this group that affect human beings [1] [2]

Nearly 70% to 80% of healthy people have antibodies against BKV and JCV [3] [4]. Initial infection with both BKV and JCV occurs commonly in childhood or in adolescence and is generally asymptomatic [5]. These viruses can persist latently in many sites, including kidneys, CNS and lymphoid cells. Recrudescence of latent infection may then occur in immunocompromised individuals [6].

The occurrence of BKV and JCV in people infected with human immunodeficiency virus (HIV) ranges from 20% - 44% [7]. In immunocompromised patients, JCV is the main etiologic agent of progressive multifocal leukoencephalopathy (PML), a fatal neurological disease, as well as granular cell neuropathy, encephalopathy and meningitis, the BKV is associated with hemorrhagic cystitis in stem cell transplantation (HSCT) hematopoietic and interstitial nephropathy in kidney transplant recipients, it has recently been shown that is associated with neurological complications [4] [8] [9].

Nonetheless, PML prevalence frequently increases during AIDS epidemics, where up to 5% of AIDS patients may develop the disease. In fact, the mortality associated with PML has also expanded from 1.5 per ten million people in the initial HIV era to 6.1 deaths per ten million persons in the post HIV era [9].

Currently, a new PML presentation has appeared among patients managed with immunomodulatory drugs for autoimmune diseases [9]-[11]. BKV is associated with hemorrhagic cystitis in hematopoietic stem cell transplantation recipients and interstitial nephropathy in kidney transplant recipients and has lately also been demonstrated to be related to neurologic exacerbations [4].

There are about 1.7 million people infected with HIV in Latin America and 250,000 in the Caribbean in Colombia in 2014. In Cordoba, 9700 cases were reported, with a mortality rate of 6.7% and 380 of these cases were notified as HIV/AIDS positive [12]-[14].

Between 1992-2000 in Brazil and Mexico, the prevalence of PML in postmortem samples of patients confirmed with AIDS was between 0.8% and 3% [15]. In Colombia, only four cases of polyomavirus infection have been reported, Triana *et al.*, [16] reported the first case of PML in Latin America and Colombia (Bogotá) associated with natalizumab in a patient with a diagnosis of multiple sclerosis; the virus was identified in brain biopsy using the technique of *in situ* hybridization. In Cali, Cadavid *et al.* [17] studied two patients with kidney transplants; BKV was identified by immunohistochemistry in a kidney biopsy. However, there are no studies in Colombia on polyomavirus in HIV individuals associated with neurologic disease.

The objective of this study was to detect JCV and BKV in HIV patients with symptoms consistent with encephalitis and/or meningitis.

## 2. Materials and Methods

### 2.1. Patients and Specimens

34 CSF specimens were collected from HIV patients with suspected meningitis and/or encephalitis between September 2009 and December 2011, at the San Jerónimo of Montería hospital main clinical center of third level of care of the department of Cordoba. 25 patients were males and 9 females aged 3 - 63 years. CSFs were frozen at  $-80^{\circ}\text{C}$  until processing. The selection of patients was independent of group age, gender and membership in health system, the selection criteria were: patients with diagnosis of suspected encephalitis [18] was defined as: fever, headache, convulsions, signs of brain involvement, such as alteration of consciousness and/or personality, seizures and focal neurologic signs and the diagnosis of suspected meningitis [19] was defined as: fever, headache, neck stiffness.

### 2.2. Molecular Detection of JC and BK Viruses

Polyomavirus DNA was extracted from 200  $\mu\text{l}$  of CSF samples using a resin column kit (Quiagen, USA Cat. 51306) according to the manufacturer's instructions. The DNA was kept at  $-20^{\circ}\text{C}$  until processing. To detect JCV and BKV a kit LightMix® polyomaviruses JC and BK (Cat-40-0203-32, Roche Diagnostics, Tibmolbiol), multiplex PCR in real-time was performed. The kit amplifies a fragment of the gene encoding t antigen 175 bp (BKV) and 172 bp (JCV). In each test negative and positive controls and internal standards were included; those were used to generate viral load curves for JCV and BKV ( $10^1$  to  $10^6$ ).

### 2.3. Laboratory Data

The CSFs were analyzed by: tests of glucose and protein levels, cell count of leukocytes and erythrocytes, Chi-

nese ink, Gram staining, Ziehl-Neelsen and culture.

## 2.4. Ethics

Institutional standard guidelines of the Minister of Health of Colombia and University of Cordoba ethics committee were followed for the collection of patient's CSF samples after obtaining their written informed consent for involvement in the study. All ethics issues were according to the resolution number 8430 of 1993 [20]. During the study confidentiality of the information was maintained.

## 2.5. Statistics

Chi-square test and ANOVA were used for statistical analysis. Analysis was performed in EpiInfo Version 7.1 (CDC, Atlanta) software.  $P < 0.05$  was considered significant.

## 3. Results

Between September 2009 and December 2011, 34 HIV patients with neurological manifestations were studied. The median age of the cohort was 33.5 years (range, 3 - 63 years); of these 34 patients 25 (74%) were male and 9 (26%) were female.

### 3.1. Clinical Features

The following clinical manifestations were found in 34 patients: 28 (82%) patients presented with headache, 20 (59%) fever, 14 (41%) nausea, 12 (35%) neck rigidity, 11 (33%) convulsions, 8 (24%) altered mental status, 7 (21%) decrease of the muscular force, 4 (12%) irritability, 3 (9%) dysarthria, 2 (6%) hypoesthesia and 1 (3%) flaccid paralysis. CSF samples showed opportunistic pathogens in 6 of 34 (18%) patients; 4 (12%) patients had *Cryptococcus neoformans* and 2 (6%) were infected by *Treponema pallidum*. The hematological and biochemical parameters were as follows: leukocyte count (average  $54.03/\text{mm}^3$ ), predominantly mononuclear (average 15%), erythrocyte count (average  $44.96/\text{mm}^3$ ), glucose levels (average 35.12 mg/dl), and increase in protein (average 130.11 mg/dl).

### 3.2. Detection of JCV and BKV in CSF

JCV was detected in 9/34 patients (26%), with a mean viral load of 1.535 copies/ $\mu\text{l}$  (range, 500 – 1,000,000 copies/ $\mu\text{l}$ ). One patient (3%) had coinfection with JCV/BKV; a viral load of 850 copies/ $\mu\text{l}$  of JCV and 650 copies/ $\mu\text{l}$  of BKV were found. Viral load, PCR and hematological and biochemical parameters are described in **Table 1**. There were no significant differences in the age, sex, clinical manifestations and patients with infection by JCV and BKV.

### 3.3. Clinical Presentation of Patients with Infection by JCV and BKV

The median age was 35 years (range, 3 - 62 years). Only one of 34 (3%) patients died with infection by JCV (**Table 1**). Patients with JCV or BKV infection presented the following signs and symptoms: headache (80%), fever (70%), seizures (40%), vomiting (40%) and nausea, decreased muscle strength, nuchal rigidity and hypoesthesia, altered mental status were seen with less frequency (**Table 1**). Ten patients showed glucose average of 40.7 mg/dl, protein levels had an average of 171.66 mg/dl, protein levels were elevated in only 3 patients. Regarding hematological patterns, the leukocytes had an average of  $19.8/\text{mm}^3$ , erythrocytes had an average of  $109.8/\text{mm}^3$ , predominantly mononuclear 10%, in 3 patients the leukocyte count was elevated (**Table 1**).

## 4. Discussion

This is the first prospective study carried out in Colombia in HIV patients infected by JCV and BKV; all HIV patients enrolled in the study had suspected meningitis or encephalitis. The overall prevalence was 26% for polyomavirus and 3% of coinfection with JCV/BKV. The frequency of infection with JCV was significantly higher than other studies, which reported infection rates of 6% with JCV and coinfections with JCV/BKV in only 3.3% (**Table 1**). In several studies carried out in urine samples, cervical carcinoma, semen and saliva, the detection

Table 1. Clinical description, viral load and CSF examination of patients with JCV and BKV.

Age/gender	Years	Symptoms	Medical condition	CAT/NMR	Viral load copies/ $\mu$ l /JCV-BKV	Glucose mg/dL	Protein mg/dL	Leukocytes $\text{mm}^3$	PMN %	MN %	Erythrocytes $\text{mm}^3$
3/F	2011	Headache, fever, diarrhea, abdominal pain, right hemiparesis	Desnutrición grade II, H1N1 virus (October 2009) diagnosed with HIV in 2008	MRI: Multimodular formation on basal ganglia in both sides	4.500/JCV	43	22.6	5	0	0	70
31/F	2009	Headache, amnesia, seizures	HIV diagnosis on admission	CAT 1: Focal injuries on brain parenchyma. HIV encephalopathy. CAT 2: Basal ganglia lesions with enhancement Brain edema	$>1.35 \times 10^6$ /JCV	54	20	1	0	0	3
*23/M	2010	Headache, fever, seizures, neck stiffness	Previous diagnosis of HIV, TBC pulmonar		900/JCV	42	237.4	80	99	1	600
39/F	2010	Headache, fever, vomiting	Previous diagnosis of HIV	CAT: Hypodense areas on frontal and parietal lobes with cerebritis. CAT with contrast: Hypodense frontal and parietal areas. Some areas with cerebritis. Brain edema	2.450/JCV	34	376.35	1	0	100	2
46/F	2010	Fever, vomiting	Positive VDRL serum, HIV diagnosis on admission	ND	500/JCV	26	13.1	5	0	100	10
62/M	2010	Decreased muscle strength, headache, hypoesthesia blurry vision, dysarthria	Diagnosed of HIV 8 years ago	Normal	2.200/JCV	73	43.8	0	0	0	0
22/M	2010	Headache, fever, seizures	Previous diagnosis of HIV	ND	500/JCV	56	34.3	15	0	100	185
36/M	2011	Fever, paresthesia in hands, myalgias, cervical adenopathy and arthralgias	Diagnosed of HIV 7 years ago	CAT: Irregular areas on convex, parietal and temporal lobes	2.300/JCV	49	20.8	0	0	0	0
47/M	2011	Decreased muscle strength, headache, hypoesthesia, fever, altered mental status, vomiting neck stiffness, and paraparesia	Diagnosed of HIV 3 years ago	NMR: hydrocephalus with trans-ependymary edema	500/JCV	13	371	88	1	99	108
34/F	2011	Decreased muscle strength, headache, seizures, vomiting, cervical stiffness, loss of vision and equilibrium, diarrhea	Diagnosed of HIV 5 years ago	CAT: prominent cisterns on frontal and temporal lobes	850 JCV/650 BKV	17	124	3	0	100	120

\*Patient died. F: female. M: male. PMN: polymorphonuclear leukocytes. MN: mononuclear leukocytes. NMR: nuclear magnetic resonance. CAT: computed axial tomography. ND: No done.

rate of JCV was 44% and 19% for BKV; coinfections were detected in up to 56% mainly in HIV patients. These data demonstrate the importance of epidemiological surveillance and the role of these viruses in neurological, renal and respiratory diseases and in cancer (**Table 2**).

The human polyomaviruses have been associated with individuals infected with HIV in whom the prevalence is higher than in healthy people [21]. This could explain the frequency of detection in the present study and its association with HIV and diseases of the CNS. However, most of the studies, including our work, analyzed samples of hospitalized patients, which may explain the high prevalence rates compared with low rates in healthy patients [22].

In African HIV patients, a rate of 6% of JCV was detected in CSF and it was not associated with PML diagnosis. PML was not evaluated in the present study because nuclear magnetic resonance (NMR) and computed axial tomography (CAT) imagines were not available; we obtained only radiological image interpretations. Nevertheless, in our patients infected by JCV/BKV, there were strong clinical presentations compatible with meningitis or encephalitis [6].

The detection of JCV in patients with meningitis or encephalitis and its causal association with these illnesses has been documented and it has been established that 2 of each 131 patients with initial diagnosis of meningitis or encephalitis are infected by JCV and they present with typical symptoms such as migraine, dizziness, nuccal rigidity and diplopia. In contrast, PML differs from polyomavirus infection, in that PML has neurological deficits, absence focal and focal injuries in the white matter of the brain. In the present study, the clinical manifestations are similar to those described in the literature for the neurological complications associated with these viruses [9].

The neurological problems arising from infection with BKV have been described extensively; meningo-encephalitis is common in immunocompromised patients [1]. In the present study, a JCV/BKV coinfection was identified in a patient with a clinical picture compatible with infection of the CNS; however, the patient did not present significant neurological complications.

With regard to the laboratory findings, the CSF of patients with infection by JCV did not show remarkable changes, although there was an increase in cell count, protein and increased glucose levels. In the present study, we observed a 33% shift in the CSF, similar to that reported by Netravathi *et al.* (2013) [23] and Berger 2014 [24].

**Table 2.** Studies on JCV and BKV in different countries.

Country	Sample	Method	Nro. of patients	Prevalence	Reference
Brasil	Urine	PCR	160 children HIV+ y 60 healthy children	25% JCV; 40% BKV	[7]
Brasil	Saliva	PCR-TR	60 children HIV+ y 60 healthy children	3% JCV; 6% BKV	[27]
Brasil	Urine	PCR	75 HIV+	19% BKV; 14.7% JCV; 56% coinfection	[4]
Italy	Urine y semen	PCR-TR	212 infertile men y 200 fertile men	34% JCV, 1% BKV in infertile; 20% JCV, 1% BKV fertile	[28]
Serbia	Urine	PCR	93 HIV+/107 healthy	44% JCV HIV+; 32% JCV healthy	[22]
Kenia	Cervical smears Cervical carcinomas	PCR	healthy women/HIV+	5% and 16% JCV HIV+, 8% and 6% JCV healthy	[29]
África	CSF	PCR/ELISA	131 meningitis and/or encephalitis	4% BKV; 2% JCV 3% coinfection	[6]
Zambia	CSF	PCR	331 adult HIV+	6% JCV	[30]
India	CSF	PCR/MRI	1.965 HIV+	3% JCV	[23]
Colombia	CSF	PCR-TR	34 HIV+	26% JCV; 3% BKV/JCV	<b>Present study</b>

In the present study for the first time in Colombia, we used a multiplex PCR for the detection in CSF samples of JCV and BKV in a HIV population with clinical suspicion of neurological disease. Molecular techniques are useful and reliable tools for the diagnosis of viral agents of clinical importance. In the case of JCV/BKV, the sensitivity of the PCR in CSF was 72% - 92% and the specificity 92% - 100%; our results thus confirm that PCR is a useful test for diagnosing polyomavirus infection [1] [25]. However, the present work has limitations in the radiological images, because several patients are often difficult to distinguish between PML and encephalopathy by HIV [26]. On the other hand, the small number of samples (34 patients), it does not allow a conclusive statistical analysis, because there were no significant differences between the groups.

## 5. Conclusion

In conclusion, in the present study, polyomavirus were detected for the first time in Colombia in HIV patients. The significant polyomavirus frequency suggests a possible association of these viruses with infections of the CNS.

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