Urinary Cytology Associated With Human Polyomavirus and Indinavir Therapy in HIV-Infected Patients

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Key Words: Urinary cytology; Hematuria; Indinavir sulfate; BK virus; Polyomavirus; Urine; HIV

Abstract

We retrospectively analyzed 155 urine cytology samples (78 from patients treated with indinavir; 77, no indinavir) from 90 HIV+ patients to evaluate possible association between human polyomavirus and hematuria and to describe indinavir-associated urinary cytologic findings. The CD4 count also was recorded. Variables studied included the presence of cellular viral changes consistent with polyomavirus infection (PVCs), microscopic hematuria, multinucleated cells, indinavir crystals, neutrophils, and eosinophils. Twenty-two samples (15.8%) from patients with CD4 counts of more than 200/µL (>200 × 106/L) showed PVCs. Multinucleated cells, of presumed histiocytic origin based on morphologic features and selective immunocytochemical findings, were present in a higher percentage of samples from indinavir-treated patients. Neutrophils were present in a higher percentage of indinavir-treated patients. Indinavir crystals were identified in 9 samples (12%) from patients receiving indinavir. The lower percentage of PVCs in HIV+ patients with high CD4 counts likely represents an indirect antipolyomavirus indinavir effect by boosting immunity. Multinucleated cells (presumably histiocytic) and acute inflammation are associated with indinavir therapy. Indinavir crystals have a characteristic fan or circular lamellate appearance. Because indinavir crystals may be associated with genitourinary disease, recognizing and reporting them is clinically relevant in HIV+ patients.

Genitourinary disease will develop in one third of HIV+ patients or those with AIDS,1 with hematuria reported in more than 25%.2 The cause of hematuria in immunocompromised patients may be related to infection with or reactivation of the human polyomavirus (PV), based on the previously reported association of the BK virus (BKV) with hemorrhagic cystitis in an HIV+ patient and in bone marrow transplant recipients3-5 and microscopic hematuria following bone marrow transplantation.6

Protease inhibitors are used commonly for the treatment of HIV+ patients. During treatment with one such drug, indinavir, cystitis and pyuria have developed. We have observed the presence of indinavir crystals and multinucleated cells morphologically suggestive of a histiocytic origin in urine samples from patients receiving indinavir therapy.

During the last several years, routine urinary cytologic studies in HIV+ patients for the evaluation of microscopic hematuria, crystals, pyuria, drug reaction, and cystitis have been done in our laboratory. We performed a retrospective study on these samples to evaluate the presence of PV cells and its possible association with hematuria and to analyze the cytologic features associated with indinavir therapy.

Materials and Methods

The archives of the Cytopathology Section, Laboratory of Pathology, National Cancer Institute, Bethesda, MD, were searched for urine samples from HIV+ patients, processed between July 1996 and June 1998. A total of 155 samples (152 voided urine and 3 instrumented urine samples) from 90 patients (82 males and 8 females) were retrieved. The CD4
cell count also was recorded. The protocols in which all patients participated were approved by the appropriate institutional review boards.

Available cytocentrifuged samples were rescreened by 2 cytotechnologists (A.M.W. and K.B.) and reviewed by 2 cytopathologists (A.C.F. and A.A.). The cytocentrifuged samples were prepared from concentrated urine samples fixed with Saccomanno fixative and stained with Papanicolaou stain. The cytologic preparations were reviewed for the presence of cellular changes consistent with polyomavirus infection (PVCs), microscopic hematuria, multinucleated cells, indinavir crystals, neutrophils, and eosinophils. PVCs are characterized morphologically by large intranuclear, basophilic, and/or pale homogeneous inclusions or a coarse chromatin network. Indinavir crystals could be identified by their characteristic fan or circular shape with a lamellate body. The presence of any RBCs was noted as microscopic hematuria. The multinucleated cells often exhibited large granular or vacuolated cytoplasm, focally containing debris, and multiple round to ovoid to bean-shaped nuclei. Immunoperoxidase staining for CD68 (DAKO, Carpinteria, CA) and cytokeratins (AE1/AE3, Boehringer Mannheim, Indianapolis, IN) was performed on selected cytocentrifuged samples following a standard avidin-biotin complex method. Statistical analysis was done using the Fisher exact test.

Results

A total of 28 samples demonstrated the presence of PVCs. In this group, 22 samples (79%) also had microscopic hematuria. Microscopic hematuria was not identified in 6 samples (21%). Cytologic examination of 127 urine samples did not reveal PVCs. In this group, 103 samples (81.1%) showed microscopic hematuria.

Six (38%) of 16 samples from patients with CD4 counts of 200/µL (200 × 10⁶/L) or less showed PVCs. Among patients with CD4 counts of more than 200/µL (>200 × 10⁶/L), PVCs were revealed by urinary cytologic examination in 22 samples (15.8%; P = .04).

Seventy-eight samples were derived from patients currently treated with indinavir, and 77 samples were derived...
from patients never treated or not currently receiving treatment with indinavir. Microscopic hematuria was present in the majority of patients with (61 [78%]) or without (65 [84%]) indinavir treatment. Multinucleated cells, of presumed histiocytic origin based on morphologic features and selective immunocytochemical findings, were present in a higher percentage of samples from patients treated with indinavir (20 [26%] vs 6 [8%]; P = .0045). Although neutrophils were present in samples from patients who had or had not received indinavir, they were present in a higher percentage of patients currently receiving indinavir (P = .0201). Indinavir crystals were identified in 9 (12%) of 78 samples from patients receiving indinavir therapy and in only 1 (1%) of 77 samples from patients not receiving indinavir (P = .0177). For this 1 sample, indinavir therapy had been discontinued in the patient 24 hours before the sample was obtained.

**Discussion**

Primary PV infection usually takes place during childhood; thus, by the age of 10 years, the prevalence of BKV antibody is nearly 100%, with virus persisting indefinitely in the renal tissue thereafter.6 During the course of immunosuppression associated with HIV infection, AIDS, or both, reactivation of latent viral infections can be the cause of substantial morbidity.10 The viruria associated with PV is not restricted to HIV+ people, but also is seen in such diverse conditions as post–organ transplantation, chemotherapy treatment, pregnancy, and diabetes. It also can be seen in people without an obvious cause for immunosuppression.7 The inoculation of BKV into laboratory animals has demonstrated its tumorigenic potential through the induction of brain neoplasms and osteosarcomas.11 BKV also has been implicated as the causative agent for reported cases of renal failure, tubulointerstitial nephritis, and disseminated infection in immunocompromised patients.12-14

In 1 series, 84 urine samples from patients after bone marrow transplant were studied by polymerase chain reaction and electron microscopy to investigate the association of PV and microscopic hematuria.6 Both methods demonstrated a significant association between microscopic hematuria and the presence of PV in the urine samples from the patients. In our study, samples from patients with and without PVCs showed a high frequency of microscopic hematuria (79% with PVCs and 81.1% without PVCs), suggesting that perhaps PV reactivation may not be an important factor in the genesis of microscopic hematuria in HIV+ patients. Nevertheless, these high frequencies of microscopic hematuria may be explained partially by our sensitive criteria for determining the presence of microscopic hematuria.

Indinavir, a retroviral protease inhibitor, is one of the medications currently used in the treatment of patients with HIV infection or AIDS. As indinavir is excreted by the kidneys and urine solubility is low, one side effect is the formation of indinavir crystals.15 In approximately 4% to 12% of patients treated with indinavir, kidney stones develop that are composed partially or completely of indinavir.8,16 Thus, the pathologic reporting of indinavir crystals in urinary cytologic samples in patients undergoing

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**Table 1**

Polyomavirus Cells and Microscopic Hematuria in 155 Urine Samples From HIV+ Patients*

<table>
<thead>
<tr>
<th>Microscopic Hematuria</th>
<th>Cellular Changes Consistent With Polyomavirus Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present (n = 28)</td>
<td>22 (79)</td>
</tr>
<tr>
<td>Absent (n = 127)</td>
<td>103 (81.1)</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage).

**Table 2**

Correlation of Polyomavirus Cells With CD4 Count in 155 Urine Samples From HIV+ Patients*

<table>
<thead>
<tr>
<th>Cell Count</th>
<th>Cellular Changes Consistent With Polyomavirus Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤200/µL (&lt;200 × 10⁹/L) (n = 16)</td>
<td>6 (38)</td>
</tr>
<tr>
<td>&gt;200 (&gt;200 × 10⁹/L) (n = 139)</td>
<td>22 (15.8)</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage). Percentages are based on the number of samples in the cell count category. Values are given in conventional units.

**Table 3**

Urinary Cytologic Features of 155 Urine Samples From HIV+ Patients Treated or Not Treated With Indinavir*

<table>
<thead>
<tr>
<th>Indinavir Treatment</th>
<th>Cellular Changes Consistent With Polyomavirus Cells</th>
<th>Microscopic Hematuria</th>
<th>Multinucleated Cells</th>
<th>Indinavir Crystals</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes (n = 78)</td>
<td>11 (14)</td>
<td>61 (78)</td>
<td>20 (26)‡</td>
<td>9 (12)‡</td>
<td>29 (37)†</td>
<td>5 (6)</td>
</tr>
<tr>
<td>No (n = 77)</td>
<td>17 (22)</td>
<td>65 (84)</td>
<td>6 (8)</td>
<td>1 (1)‡</td>
<td>15 (19)</td>
<td>6 (8)</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage).

‡ Statistically significant difference (P < .05).

† Indinavir treatment discontinued 24 hours before the sample was obtained.
current or previous indinavir therapy is clinically significant. In the present study, indinavir crystals were present in 12% of samples (9/78) from patients receiving indinavir therapy. Aggregates of needle-shaped crystals, such as uric acid, tyrosine, sulfa, and bilirubin crystals, may mimic indinavir crystals. Unlike indinavir crystals, uric acid crystals show diverse morphologic features, including needle-, spear-, star-, rosette-, barrel-, and whetstone-shaped crystals and rhombic and 6-sided plate-shaped crystals.17 Tyrosine crystals display a fine, silky appearance; when in clusters, they assume sheaf or rosette forms.17 Clusters of needles, granules, or both characterize the appearance of bilirubin crystals in urinary cytologic samples.17 Although sulfonamide crystals may be fan-shaped, they exhibit polymorphous morphologic features like uric acid crystals, with forms ranging from rosettes to shocks of wheat, hexagons, whetstones, well-formed plates, and granules.17

Urinalysis revealed persistent pyuria in some of our patients, and cytologic evaluation of urine samples was requested. Cytologic samples from patients receiving indinavir therapy showed a higher frequency of acute inflammatory cells and multinucleated cells of histiocytic origin compared with samples from patients without a history of indinavir therapy. These findings suggest that pyuria and multinucleated cells of histiocytic origin may be related to indinavir therapy in many patients.

Another interesting observation is that a lower percentage of PVCs were present in patients with CD4 counts of more than 200/μL (>200 × 10^6/L). This observation may represent an indirect anti-PV action of indinavir. It is likely that indinavir therapy decreases the HIV viral load, promoting an increase in the number of CD4 cells and allowing the organism to respond more efficiently against viral infections. Indinavir previously has shown an antiviral effect against a DNA virus through the clearing of human herpesvirus 8 from peripheral blood mononuclear cells in an HIV+ patient.18

Our findings suggest that PV is more common in HIV+ patients with low CD4 counts. Also based on our observations, multinucleated cells, presumably of histiocytic origin, and acute inflammation are associated with indinavir therapy. Indinavir crystals may lead to the development of clinically significant genitourinary disease, which most certainly makes the recognition and reporting of indinavir crystals in urinary cytologic samples from HIV+ patients clinically relevant.

References


