Role of Urinary Cytology in Detecting Human Polyoma BK Virus in Kidney Transplant Recipients

A Preliminary Report

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Key Words
Urine cytology • BK virus • Renal transplant recipients

Abstract
Objective: To report data on the evaluation of the efficacy of urine cytology in detecting BK virus (BKV). Material and Methods: Papanicolaou-stained cytospin preparations of randomly collected urine samples from 8 renal transplant (RTR) patients (5 males, 3 females, age: 23–63 years) who previously tested positive for BKV by PCR in urine or blood or both were studied. Urine sediment from 3 of the patients was processed for ultrastructural examination (EM). Renal biopsies before and after BKV detection were also evaluated. Results: The interval between renal transplant and urine cytology ranged between 2 and 5 years. Urine from females was difficult to assess due to vaginal contamination. In 2 of the 5 urine specimens from male recipients BKV-infected decoy cells were identified. Viral particles suggestive of BKV were identified on EM in 2 of the 3 specimens studied. Kidney biopsies showed morphologic features suggestive of BKV infection in 2 cases, 1 each detected on cytology and EM, respectively. Conclusion: Screening for the presence of decoy cells in urine provides a simple sensitive means for the diagnosis of BKV nephropathy in RTR.

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Introducion

BK virus (BKV) is a human polyoma virus associated with a range of clinical presentations. Primary BKV infections occur in early childhood and the virus remains latent in the kidney and in B lymphocytes. Renal transplant recipients (RTR) are at risk of reactivation of BKV. When reactivated, the virus proliferates within the nuclei of renal tubular and uroepithelial cells with a possible severe derangement of renal function and loss of allograft function [1]. BKV nephropathy is characterized by tubular changes often associated with interstitial inflammation and tubulitis with the tubular cell nuclei heavily altered by viral inclusions [2, 3]. The disease occurs in 2–9% of RTR and is correlated with the shedding of a large number of virus-infected cells in the urine and with the presence of BKV DNA in the serum [4]. The hallmark of BKV infection in the urine is the presence of ‘decoy cells’ [3–6]. These cells are readily identified by Papanicolaou stain on fixed urine specimens. The optimal method for monitoring polyoma virus infection in RTR has not been defined [7]. In clinical practice the cytologic evaluation of urine samples is widely used for the diagnosis of BKV infection [6–8].

This brief communication evaluates the utility of urine cytology in detecting BKV in urine from RTR tested positive for BKV by polymerase chain reaction (PCR); it also correlates results with transplant biopsy specimens and
documents if electron microscopic examination (EM) of urine sediment further augments BKV detection.

**Material and Method**

The study included 8 RTR (5 males, 3 females; age 23–63 years). Papanicolaou-stained cytospin preparations of randomly voided urine from the 8 RTR under follow-up in the Hamad Al-Essa Organ Transplant Centre, Kuwait were studied. All 8 had urine positive for BKV by nested version of PCR assay using primers (Hybaid, USA) targeting the T region of the virus and in 5 of them the blood also tested positive for BKV by PCR (table 1). Renal biopsies either before and/or after BKV detection in urine by PCR were also evaluated for morphologic evidence of BKV. Urine sediment from 3 of the 5 male patients was processed for EM.

**Results**

The interval between renal transplantation and urine cytology ranged between 16 and 54 months. BKV nephropathy was diagnosed 16–54 months after transplantation and all subjects had a rise in serum creatinine compared to borderline at the time of diagnosis (table 1).

**Urine Cytology**

In 2 of the 5 urine specimens from male RTR decoy cells suggestive of viral replication were detected. Cytopathic changes appeared as dense, gelatinous or granular basophilic inclusions often occupying most of the nucleus, with no definite surrounding halo (fig. 1A). Often a rim of granular chromatin was seen around the inclusion (fig. 1B). Occasionally infected cells display a large smudgy area of dense chromatin with a rim of clumped nuclear material (fig. 1C). The cytoplasm of many of the epithelial cells appeared vacuolated and foamy. In 2 of the 3 cases EM revealed 51 nm intranuclear and cytoplasmic particles consistent with BKV. One case (case 2) was pos-

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**Table 1. Correlation of urine cytology, histology, EM and BKV detection by PCR**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Post-transplant months</th>
<th>Urine BKV by PCR</th>
<th>Urine cytology</th>
<th>Histology</th>
<th>EM</th>
<th>Blood BKV by PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>M</td>
<td>22</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>M</td>
<td>19</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>F</td>
<td>16</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td>24</td>
<td>+</td>
<td>VC</td>
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<td>-</td>
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<td>25</td>
<td>M</td>
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<td>52</td>
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<td>VC</td>
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<td>+</td>
</tr>
</tbody>
</table>

VC = Vaginal contamination; ND = not done; NC = no cells.
itive for the virus on cytology and EM (table 1). In females (3 cases) vaginal contamination made it difficult to evaluate for the presence of virus.

**Histopathology**

Tubulointerstitial infiltrates and marked tubulitis were seen in kidney biopsies in association with reactive/degenerative tubular epithelial changes. The tubular epithelial cells displayed moderate to marked anisocytosis and hyperchromasia with nuclear cytoplasmic inclusions (fig. 1D, E).

**Discussion**

Polyoma virus (JC and BK) are widely latent in human beings [7]. RTR receiving immunosuppressive therapy have a 10–60% chance of reactivation of polyoma virus accompanied by shedding of urothelial cells [9]. Viral shedding is not consistently associated with dysfunction. The reported prevalence of BKV is 1.9–4.5% in RTR with a graft loss exceeding 30% [10]. BKV nephropathy mimics acute tubular necrosis or rejection clinically and may present between 2 and 60 months (median 9) after transplantation [1, 3]. The final diagnosis rests on identifying BKV.

BKV can be detected in the urine, plasma and biopsy tissue of patients. Currently, there is no consensus regarding the laboratory technique best suited for clinical monitoring [7]. Urine cytology, a technically simple procedure [3, 8], has limited capability to predict clinical disease as it has been found to be associated in only 28% of cases [1, 2]. PCR assays for BKV DNA can be performed on plasma, urine and tissue with a sensitivity of 100% and a positive predictive value of 85% proving to be a valuable clinical tool for the detection of BKV [4]. When urine is collected concurrently with an allograft biopsy performed for rising serum creatinine the positive predictive value of urine cytology for the diagnosis of viral nephropathy is 90% [2]. Coleman et al. [5] found that only 6.7% of the patients had large numbers of inclusions although 29% of patients excreted the virus. In our study inclusions were identified in 25% of the cases that excreted the virus.

In renal biopsy specimens it is often difficult to differentiate between the effects of viral pathology on the tissues and the changes caused by acute rejection. Sophisticated immunohistochemical and in situ hybridization can confidently clinch the diagnosis. Due to interlaboratory variability observed in qualitative PCR techniques it may be desirable to have internal standards or reproducible, quantitative PCR techniques or other molecular tests to monitor BKV load [1]. The advantages and disadvantages of different modalities of BKV identification have been beautifully discussed by Lin et al. [4].

EM of urinary sediment failed to show viral inclusions if the urine sample was negative for signs of polyoma virus by light microscopy [2]. However, as seen in our study, De Las Casas et al. [8] also found one urine specimen with negative urine cytology to be positive on EM for the virus.

**Conclusion**

Routine cytologic evaluation of urine sediment appears to be a sensitive method to determine the presence of BKV nephropathy in RTR.

**References**