Molecular Detection of BK Polyomavirus in Patients Under Hemodialysis

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Abstract

The study was conducted in Kirkuk city-Iraq from of February 2018 to September 2018. The number of chronic kidney disease patients under study were 60 patients whose ages were between 40-70 years old. These patients admitted to Kirkuk general hospital (hemodialysis unit). The control group who were matched to the patients studied, included 30 healthy blood donor and their ages were between 30-75 years old. Blood samples were collected for detection of BK polyomavirus DNA by real time-PCR. The study showed that the highest rate of BK polyomavirus DNA occurrence were recorded in patients with chronic kidney disease when compared with control group (51.66% versus 3.33%) (P: <0.01). The study showed that the highest mean level of IL-6 was recorded in patients with chronic kidney disease comparing with the control group (78.42 v.s. 3.40 ng/ml). The result was highly significant. The present study demonstrated that the highest mean level of IL-6 was found in prostitutes cancer patients who were positive to BK polyomavirus DNA comparing with BK polyomavirus DNA negative (52.54 versus 25.88 ng/ml)

It was concluded that BK polyomavirus was frequently detected in chronic kidney disease patients and could play a relevant role in the development and progression of human hemodialysis.

Keyword: Hemodialysis; BK polyomavirus, CKD

Introduction

BK virus (BKV) belongs to genus Polyomavirus within the Polyomaviridae, a family of small, nonenveloped, double-stranded DNA viruses (1). Its genome is divided into 3 regions: the early, late and transcriptional control region (TCR). Early genes code the regulatory small and large T proteins and late genes code the viral capsid proteins (VP1, VP2, and VP3) and agnoprotein [1-3]. The major prognostic factor of BK virus nephropathy is the stage of the disease at the time of diagnosis. When documented in advanced stage with associated chronicity changes, it has worse prognosis with a renal survival at 12 months from 10 to 40%. In contrast, an early stage diagnosis with only viral cytopathic changes, survival is over 80% at 12 months [5-6]. The primary infection with BKV usually occurs in early childhood. It is estimated that 35-90% of the general population will acquire the primary infection during infancy and its seroprevalence reaches 46%-94% in adults depending on the studied regions [7-9]. Primary infection is usually asymptomatic, but after initial infection, BKV may persist lifelong in the kidney and genitourinary tract epithelium and possibly peripheral blood mononuclear cells, tonsils and other hematopoietic tissues [10,11]. Since the pre-transplant status affects the renal transplantation success and ultimately the survival rate, identifying the probable risk factors that increase the chance of BK virus replication in end-stage renal disease patients can be included in
proposing proper surveillance guidelines during pre and post-transplantation\textsuperscript{[12,13]}. The aim of the study was to study the relation of BK polyomavirus (BKPyV) in hemodialysis.

**Material and Method**

The study was conducted in Kirkuk city-Iraq from February 2018 to September 2018. The number of chronic kidney disease patients under study were 60 patients whose ages were between 40-70 years old. These patients admitted to Kirkuk general hospital/Hemodialysis unit. The control group who were matched to the patients studied, included 30 healthy blood donor and their ages were between 30-75 years old. Blood samples were collected (3ml EDTA and 2ml for serology) for detection of BKPyV DNA by real-time-PCR and the level of IL-6 by immunefluorescent technique.

**DNA extraction**

DNA was extracted from all prostate samples by lysis and proteinase K digestion in EDTA, and 0.1% sodium dodecyl sulfate (SDS), the samples were extracted with phenol chloroform and precipitated with ethanol. DNA concentration and purity was determined by spectrophotometry at $\lambda=260/280$nm. The extraction process was performed in an area that was BKV free while great care was taken during the tissue sectioning procedure in order to avoid any contamination. Sectioning of the tissues was carried out using a clean microtome and a separate new blade in each case, as well as clean gloves and forceps. The sections were placed in autoclaved DNAse-free microtubes for the DNA isolation procedure.

**Real time PCR**

The BKPyV Real-TM Quant kit (SaCycler Biotechnologies) is a Real-Time test for the Qualitative and Quantitative detection of BKPyV in the biological materials. DNA is extracted from samples, amplified and detected using fluorescent reporter dye probes specific for BKPyV DNA.

![Figure 1: Real-time curves of BKPyV DNA detection](image-url)
**Statistical analysis** : Computerized statistically analysis was performed using Mintab ver 18.0 statistic program. Comparison was carried out using Chi-square ($X^2$) for determination of the $P$ value.

**Findings**

The study showed that the highest rate of BKPyV DNA occurrence were recorded in patients with chronic kidney disease when compared with control group (51.66% versus 3.33%) ($P: <0.01$), Table 1.

<table>
<thead>
<tr>
<th>BK viral DNA (RT-PCR)</th>
<th>HD patients</th>
<th>Control group</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Positive</td>
<td>31</td>
<td>51.66</td>
<td>7</td>
</tr>
<tr>
<td>Negative</td>
<td>29</td>
<td>48.33</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100</td>
<td>30</td>
</tr>
</tbody>
</table>

The study showed that the highest mean level of IL-6 was recorded in patients with chronic kidney disease comparing with the control group (78.42 v.s. 3.40 ng/ml). The result was highly significant, Table 2.

<table>
<thead>
<tr>
<th>IL-6 level (pg/ml)*</th>
<th>HD patients</th>
<th>Control group</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>60</td>
<td>30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean</td>
<td>78.42</td>
<td>3.40</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SD.</td>
<td>12.745</td>
<td>1.59</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

The present study demonstrated that the highest mean level of IL-6 was found in prostates cancer patients who were positive to BKPyV DNA comparing with BKPyV DNA negative (52.54 versus 25.88 ng/ml), Tale 3.

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<th>IL-6 level (pg/ml)</th>
<th>BKPyV DNA (RT-PCR)</th>
<th>P. value</th>
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<td>12.745</td>
<td>1.59</td>
</tr>
</tbody>
</table>

Table 1: Detection of BKPyV DNA in chronic kidney disease patients and healthy control

Table 2: Serum IL-6 levels in patients with chronic kidney disease and the control group.

Table 3: Frequency of BKPyV DNA in chronic kidney disease patients according to IL-6 level
Recent studies have confirmed that chronic hemodialysis could raise the chance of BK virus replication in ESRD patients as compared to control group [11-15]. Nevertheless, we previously demonstrated that prolonged pre-transplant dialysis could be a potential risk factor for shedding of BK virus into urine in renal transplant recipients during post-transplantation [16,17]. Therefore, it can be concluded that chronic hemodialysis is a risk factor for reactivation of BK virus pre and post-transplantation.

In the present study, we assessed the potential predictive factors for development of BK virus viremia during hemodialysis period among end-stage renal disease patients. Overall, 192 ESRD patients who were under hemodialysis were enrolled without preliminary screening. The number of patients enrolled in our study was higher than previous studies, which can be helpful to gain more accurate and reliable results. The prevalence of BK viremia was 7.3% among our patients. The prevalence of BKV infection in patients with ESRD under hemodialysis or peritoneal dialysis has been reported from 0 to 33.3% in various studies [18,19]. These differences can be due to the difference in the number of studied patients, the duration and frequency of dialysis and the difference in the sensitivity of the PCR method used to identify BK virus. In general, deliberate or non-deliberate immunosuppression can cause the reactivation of BK virus. Hence, high incidence rate of BK virus infection among patients who received corticosteroid drugs is not surprising [20,21]. There are contradictory reports on the presence of BKV DNA in urinary tract tumors: the authors of one study detected BKV DNA using PCR in 31 of 52 samples, whereas other authors were unable to find it [17]. An other study detected BKV in 4 of 30 fresh tissue prostate samples and were unsuccessful with archival specimens, and observed the virus in atrophic lesions in prostate tumors. One of the most important proteins for BKV is the replicational regulatory protein, the large T-antigen that binds onto the tumor suppressor proteins p53 and pRb1, inhibiting their functions and leading to a variety of transforming effects. Given the low frequency of either TP53 or RB1 mutations in hemodialysis, it was intriguing to investigate the prevalence of BKV in prostate tumor samples [23].

**Conclusions:** BKV was frequently detected and could play a relevant role in the development and progression of human hemodialysis

**Conflict of Interest:** non

**Source of Findings:** self findings.

**Ethical Clearance:** This research was carried out with the patient’s verbal and analytical approval before the sample was taken. According to this approval, all the samples were collected and the tests were carried out. A copy of the results of the tests was then given to the patients

**References**

6. J. Levican, M Acevedo, O Leon, A Gaggero, F Aguayo, Role of BK human polyomavirus in


