Association of Polyomavirus BK Virus with Chronic Renal Failure patients

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Abstract

Background: Patients in end-stage renal disease (ESRD) were considered immunocompromised, especially those on hemodialysis (HD) procedure which seemed to produce alterations of the immune status. BK polyomavirus (BKV) was found to be an aggravating factor of renal failure, and interest in immunosuppression has increased due to BKV infection.

Objective: This study aimed to investigate the possible association between BKV with ESRD with and without dialysis.

Subjects and Methods: A case-control study included 150 serum samples collected from 50 patient with ESRD on dialysis, 50 without dialysis and 50 apparently normal subjects with normal renal function as control. Quantitative Real time PCR (RT-PCR) was done for detection of BKV viral load.

Results: Twenty (40%) out of the 50 dialysis patients were positive for BKV by RT-PCR and, eight (16%) were positive in patients without dialysis, while none of the controls was positive. There were highly significant differences (p<0.001) on comparing between the mean viral load in patients who had dialysis was 2.16X10^5 copies/ml of BK viral DNA, and those without dialysis 1.22X10^3 copies/ml of BK viral DNA.

Conclusion: These results strongly indicated the role of BKV infection (quantitatively and qualitatively) in ESRD particularly in patients with dialysis; this put dialysis as a potential risk factor for BKV infection in those patients.

Key Words: BK polyomavirus, RT-PCR, ESRD, dialysis


Introduction

BK polyomavirus (BKV) is a non-enveloped DNA virus of the polyomaviridae family that causes an interstitial nephritis in immunosuppressed patients. BKV nephropathy is now a leading cause of chronic kidney disease (1). This virus is widespread, and manifestations of infection vary with the immunologic and hematologic status of the host (2). The first human polyomaviruses were isolated from immunosuppressed patients (3). BKV takes its name from the initials of the first patient in whom it was isolated (2). BKV
causes an interstitial nephritis in kidney transplant patients, but has also been reported to cause renal disease in non-renal solid organ transplant (NRSOT) patients and bone marrow transplant recipients(4).

Primary BK infection is mainly asymptomatic or results in a mild respiratory illness (5). The natural route of transmission is not well established. Sero-prevalence studies indicated a high exposure rate to BK during childhood, with antibodies being detected in >50% of children by the age of 3 and >90% by the age of 10 (6,7). Due to the presence of viral DNA in tonsillar tissue, transmission is thought to occur via a respiratory route (5,8). There is also evidence for other possible routes of transmission such as fecal–oral, urino-oral and transplacental transmission, and via blood transfusion (9).

The role for BK infection is based on the association of renal findings with viral infection, positive serology, and identification of the viral genome in the glomerulus (3). Infection is occasionally, especially in adults, associated with chronic kidney diseases (CKD) and ESRD (4,10). The clinical significance of BKV infection in renal failure may be underestimated; the infected individuals may develop persistent viremia as a result of a dysfunctional immune response (11,12). The link between BKV infection and glomerular disease has been suggested from numerous case reports that describe onset of nephritis or nephritic syndrome after onset of BKV infection (13,14).

To best of our knowledge, few studies have been conducted on polyomaviruses in Iraq especially on kidney-transplanted recipients (15-18).

**Subjects and Methods**

The study was conducted from February 2015 to June 2016. The patients were collected from Al-Imamian Al-Khademian Medical city, Baghdad Medical city and Al-Karma hospital.

The study was performed on 100 patients with ESRD, 50 of them were on dialysis (22 females and 28 males), and 50 patients without dialysis (27 females and 23 males) kept on conservative measures, and 50 apparently healthy persons with normal renal function served as control group (24 females and 26 males). Their ages (patients and controls) ranged from (17-88) years. Informed consent was taken from all subjects enrolled in the study, the study was approved by the ethical committee in the ministry of health. Two ml of venous blood EDTA tube were taken from all subjects included in the study, from which plasma was separated and subjected for viral DNA extraction according to manufacturer instructions by using Bosphore® Viral DNA Extraction Spin Kit (Anatolia Geneworks, Turkey). The DNA extraction method is based on the silica membrane column separation. BKV viremia was evaluated by using (Bosphore® BKV Quantification Kit v1) (Anatolia Geneworks, Turkey), which is based on the principle of taqman probe.

Bosphore® BKV Quantification Kit v1 detects and quantitates the four main genotypes of BK Virus DNA in human plasma. A region within the large T-antigene (LT) encoding gene is amplified and fluorescence detection is accomplished using the FAM filter. While the fluorescence signal generated by the internal control was detected using Cy5 filter.

**Statistical analysis:** Continuous variables were expressed as mean ± SD and analyzed with one way analysis of variance (ANOVA) was used to evaluate differences of means between groups. Binomial variables were expressed as frequency and percentages and analyzed with chi square test. Correlations between HOMA-IR, other parameters were analyzed by Pearson’s correlation. P<0.05 was accepted as statistically significant.

**Results**

The current study demonstrated no statistically significant difference in the mean age among the three groups, that’s to say they are comparable in age. And also comparable regarding gender type distribution. Quantitative real time PCR analysis of BKV showed that (40%) 20 out of the 50 patients on dialysis, and (16%) 8 out of the 50 non-dialysis patients had positive BKV viremia. While none of the control group had positive BKV, P≤0.001, table (1), figure (2) and (3).

The Relative risk indicator showed that dialysis and without dialysis patients were more susceptible for infection than in control. Results of this study showed highly significant differences (p<0.001) on comparing between the mean viral load in patients who had dialysis (2.16x10^5) Copies/ ml of BK DNA and those without dialysis (1.22x10^3) Copies/ml of polyomavirus DNA, table (2).

Finally, there was significantly higher mean viral load in those patient who were on dialysis for more than 6 months than those who were on dialysis within less than 6 months (p<0.001), table (3).
Table (1) Polyomavirus Detection by q-RT-PCR in the studied groups.

<table>
<thead>
<tr>
<th>PCR</th>
<th>Study groups</th>
<th>Control</th>
<th>Dialysis</th>
<th>Without dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive%</td>
<td>0(0 %)</td>
<td>20(40%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative%</td>
<td>50(100%)</td>
<td>30(60%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p value</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Relative Risk</td>
<td>2.351</td>
<td>2.163</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% confidence interval</td>
<td>1.842 to 3.002</td>
<td>1.737 to 2.693</td>
</tr>
</tbody>
</table>

Figure (1) Real time PCR standard curve with positive polyomavirus DNA infection, the squares are the standards and the triangles are the unknowns.

Figure (2) Amplification profiles of BKV QPCR results; positive sample (over threshold), negative sample (on the line and under threshold), each one with internal control.
Table (2): Association between the mean viral load in CKD who had infection with and without dialysis of the studied group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without dialysis</th>
<th>With dialysis</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean of viral load (Copies/mL)</td>
<td>1.22 x10^3</td>
<td>2.16 x10^3</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>1.17 x10^6</td>
<td>3.15 x10^6</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>1.9 x10^7</td>
<td>1.08 x10^7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table (3): Association between the mean viral load in CKD who had less than six months of dialysis and those with more than six months

<table>
<thead>
<tr>
<th>Parameter</th>
<th>&lt;6 months</th>
<th>&gt;6 months</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean of viral load (Copies/mL)</td>
<td>2.12 x10^7</td>
<td>2.20 x10^7</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>2.32 x10^9</td>
<td>3.18 x10^9</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>1.09 x10^4</td>
<td>1.21 x10^7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Discussion

Incidence of BK polyomavirus (BKV) viremia, a major risk factor for nephropathy, among patients undergoing chronic haemodialysis remains poorly investigated. This case-control study evaluated the incidence and the risk of BKV infection among these patients.

The current study investigated the possible association between BK polyomavirus viremia and ESRD with and without dialysis and the results showed a highly significant association between Hemodialysis and BK viremia (40%) which is in line with many recent studies that focused on BK virus and ESRD on hemodialysis (19-21).

Patients in end-stage renal disease undergoing renal replacement treatment (ESRD-RRT) were considered immunocompromised. Hemodialysis or peritoneal dialysis procedures seem to produce alterations of the immune status (19).

In addition, these results suggest that pre-transplant viral status should be considered as an important risk factor for post-transplant BKV replication. Therefore, pre-transplant BKV infection screening in kidney transplant patients should be performed for improving planning of personalized immunosuppressant schemes and specific post-transplant surveillance (21).

The reasons for Polyomavirus may be an important pathogen in ESRD patients is that BKV remains latent in the kidney for the life of the host and under some circumstances, when immunity is impaired, the dormant viruses begin to replicate in the epithelial cells of the kidney, ureter and bladder (22-24). Also, because BK virions entering the renal tubular cell in smooth vesicles, aggregating and then using tubule-vesicular networks to gain access to para-nuclear areas and the nucleus to optimize infection replication and new virion assembly and shedding(25-26).

With these results, we can speculate that BKV infection in ESRD-RRT patients is linked to factors involved in the uremia-related immune dysfunction,and the mechanisms of RRTs may be needed to be changed, peritoneal dialysis is an option that could be associated with a better transplant outcome for patients undergoing kidney transplantation (19).

Conclusions Subjects on hemodialysis may be at increased risk of nephropathy due to increased incidence of BK virus reactivations and may require optimization of RRT.

References