Prevalence of BK virus among renal transplant recipients in a tertiary care hospital in Sri Lanka

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(Index words: BK virus, Polyomavirus, nephropathy, Sri Lanka, renal transplantation)

Abstract

Introduction BK virus nephropathy (BKVN) is an important cause of graft failure in post renal transplant patients. Detection of BK virus replication early enables prevention of BK virus nephropathy. Understanding BK virus epidemiology in post renal transplant patients will be useful in implementing a routine screening programme.

Objectives Objectives were to determine the prevalence of BK virus viruria and viraemia among post renal transplant patients within the first two years of transplantation.

Methodology A hospital-based, descriptive cross-sectional study was conducted on 136 clinic and in-ward patients. Plasma and urine were tested for BK virus DNA using real time PCR. Serum creatinine done within two weeks of data collection was recorded.

Results The prevalence of BK virus viruria was 53.67% and viraemia was 11%. Viraemia >1000 copies/ml was associated with abnormal serum creatinine and higher median serum creatinine. No similar association was observed with viruria. Among patients with normal serum creatinine, virus was not detected in urine in 48.9% and plasma in 92.7%.

Conclusions The prevalence of BK virus is high in this study population. Significant viraemia was associated with elevated serum creatinine. Viruria or viraemia was not detected among a large number of patients with normal serum creatinine.

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Introduction

BK virus (BKV) is a DNA virus belonging to the family *polyomaviridae*. It commonly causes mild infections during childhood. Following primary infection, the virus becomes latent primarily in the uro-epithelium.

Spontaneous reactivation of virus can occur in immunocompetent hosts but is commoner during immunosuppression. The most serious complication due to BK virus reactivation among renal transplant recipients is BK virus associated nephropathy, occurring in 1-10% of renal transplant recipients [1]. Progressive BK virus associated nephropathy may lead to graft failure and graft loss in 10-100% of cases [2].

BK virus associated nephropathy is typically diagnosed within the first year post-transplantation, although approximately 25% of the cases are seen later [2]. BK virus associated nephropathy lacks any specific clinical features and is characterized by rising serum creatinine levels, absence of histological evidence of graft rejection and presence of obstructive uropathies as hydro-nephrosis and or hydroureter [2]. BK virus associated nephropathy should be clinically suspected in patients with unexplained rising serum creatinine. Due to lack of clinical clues, early detection of viral replication is crucial to prevent progression of infection to BK virus associated nephropathy. Quantitative real-time polymerase chain reaction (PCR) assay for BK virus in urine and blood are the commonest screening tools, however universal cut-off points are not yet established. Urine BK virus load $>10^7$copies/ml or plasma BK virus load $>10^4$copies/ml are used commonly as surrogate markers for BK virus associated nephropathy. Definitive diagnosis of BK virus associated nephropathy could be made only by an allograft biopsy using histopathology and immunohistochemistry. Reduction of immuno-suppression is the corner stone of management of BK virus associated nephropathy although it always carries a risk of graft rejection. Due to the lack of successful therapeutic options, implementation of preventive strategies plays a key role in preventing graft loss due to BK virus infection. In Sri Lanka the number of kidney transplantations performed are increasing each year. Infectious complications as BK virus associated nephropathy may increase the healthcare costs and requirement for resources, which could be very challenging. Knowledge about the epidemiology of BK

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virus infection is important in making policy decisions regarding screening for BK virus reactivation among these patients. The aims of this study were to describe the prevalence of BK virus viruria and viraemia among the study population and the associated factors.

Methodology

Study design

Hospital based descriptive cross-sectional study was carried out on all post renal transplant patients within two years of transplantation, who were in-ward patients or attending clinics of University Medical Unit and National Renal Transplantation Unit, National Hospital of Sri Lanka. All consenting patients were enrolled consecutively, from July to October 2015. Patients on heparin were excluded.

DNA extraction

DNA was extracted from both plasma and whole urine using the QIAmp® Viral RNA minikit by QIAGEN according to the manufacturer's instructions.

Real-time PCR assay

BK virus real-time PCR was carried out using the Altona Real-star® BK VIRUS assay according to the manufacturer's instructions. The kit has an analytical sensitivity of 0.712 copies/µl (95 % CI 0.404 - 1.693 copies/µl), linear range 1.00E+09 to 1.00E+00 copies/µl.

Data collection

Data was collected using a pre-tested interviewer administered questionnaire. Clinical data included the exact period after transplantation, immunosuppressive treatment and recent serum creatinine done within two weeks of data collection.

Data analysis

Viriuria and viraemia were defined as detection of virus in urine and plasma respectively. Data was categorized according to the cut-points given in table 1 [1].

Table 1. Categorization of patients according to detection of virus in urine/plasma

Category	*Urine*	*Plasma*
Virus not detected	Aviruric	Aviraemic
Virus detected	Viruric (<2500 copies/ml)	Viraemic (<1000 copies/ml)
Significant viruria/viraemia	Viruria ≥ 2500 copies/ml	Viraemia ≥1000 copies/ml

Abnormal serum creatinine was defined as >1.5 mg/dl for both sexes.

Statistical analysis

Patients with different amounts of viruria and viraemia were compared using non parametric tests. Categorical variables were analysed using chi-square test with analysis of adjusted residual values. Statistical analysis was done using SPSS 21.0 (Illinois USA).

Ethical issues

Ethical approval was obtained from the Ethics Review Committee, Medical Research Institute, Colombo (16/2014). Written informed consent was obtained from all patients.

Results

Among the 136 participants 59.6% were male (Table 2). The mean age was 40.65 years (SD 14.2). Majority of the patients (n=104; 76.5%,) were on triple immunosuppression with prednisolone, mycophenolate mofetil and tacrolimus.

Earliest time to detect significant viruria and viraemia was one month post transplantation. The median post transplantation time for virus detection was 8 months in urine (IQR 3.38-13.5) and plasma (IQR 4-24).

Table 2. Characteristics of the post renal transplant patients

	Number (%)
Age (years)	40.65 (SD 14.2)
Gender	
Male	81 (59.6)
Female	50 (40.4)
Setting	
In-patient	19 (13.9)
Clinic	117 (86.1)
Triple immunosuppression	104 (76.5)
Mean daily dose	
Prednisolone	10.4mg (SD 6.7)
MMF	1397.63 mg (SD 282.34)
Tacrolimus	5.24 (SD 1.58)
Elevated serum creatinine (>1.5mg/dl)	35 (26.7)
Normal serum creatinine (≤1.5 mg/dl)	96 (73.3)

BK virus viruria

BK virus was detected in the urine in 73 (53.7%) patients. Among the viruric patients, 36 (26.5%) had viruria <2500 copies/ml and 37 (27.2%) had viruria ≥ 2500 copies/ml; seven had viruria $\geq 10^7$ copies/ml. The median urine viral load among viruric patients was 2794.5 copies/ml (IQR 244.3 - 260,000).

BK virus viraemia

Viraemia was detected in 15 (11%) patients. Among them 11 (8%) had significant viraemia with ≥1000 copies/ml including two patients with viraemia $>10^4$ copies/ml. Median plasma viral load among viraemic patients was 3038 copies/ml (IQR 678 - 6023). Both patients with viraemia $>10^4$ copies/ml had viruria $\geq 10^7$ copies/ml. All patients except one with viruria $\geq 10^7$ copies/ml, had viraemia >1000 copies/ml. The remaining patient had 462 copies/ml.

Association between serum creatinine and urine and plasma viral load

Elevated serum creatinine was detected in 35 out of 131 patients. Median serum creatinine was compared according to viruria and viraemia load (Table 3). There was no statistically significant difference in median serum creatinine in the according to amount of viruria (p=0.07). However, median serum creatinine was associated with the amount viraemia (p=0.013). Post hoc analysis showed that the significant difference was between the 1st and the 3rd groups (p=0.022).

Table 3. Median serum creatinine levels according to amount of viruria

BK virus load	*Median serum creatinine level mg/dl (IQR)*
Urine	
Aviruric	1.11 (0.93-1.4)
<2500copies/ml	1.00 (0.95-1.49)
≥ 2500 copies/ml	1.2 (0.98-1.39)
Plasma	
Aviraemic	1.08 (0.93-1.36)
<1000 copies/ml	1.35 (1.10-1.48)
≥1000 copies/ml	1.38 (1.2-1.48)

No significant association between normal and abnormal serum creatinine and amount of viruria (Table 4).

Table 4. Normal and abnormal serum creatinine according to amount of viruria

	Aviriuric Number (%)	*Viraemia <2500 copies/ml Number (%)*	*Viraemia ≥ 2500 copies/ml Number (%)*	*Total*
Normal serum creatinine	47 (74.60)	23 (71.87)	26 (72.22)	96
Abnormal serum creatinine	16 (25.40)	9 (28.13)	10 (27.78)	35
Total	63	32	36	131

A significantly higher proportion of patients with viraemia ≥1000 copies/ml had abnormal serum creatinine, compared to those who were aviraemic (adjusted residual values) (p=0.015) (Table 5). Both patients who had high level of viraemia (>10000 copies/ml) had abnormal serum creatinine.

Table 5. Normal and abnormal serum creatinine according to viraemia categories

	Aviriuric Number (%)	*Virus detected Number (%)*	*≥1000 copies/ml Number (%)*	*Total*
Normal serum creatinine	89 (76.73)	3 (75.0)	4 (36.36)	96
Abnormal serum creatinine	27 (23.27)	1 (25.0)	7 (63.64)	35
Total	116	4	11	131

Background viral loads in patients with normal serum creatinine

Urine BK virus was not detectable in 47/96 (48.9%) patients with normal serum creatinine. The median urine viral load was 9.9 copies/ml (IQR 0-2887 copies/ml). BK virus was not detected in plasma in 82 (92.7%) patients and the values in viraemic patients were beyond the 75th percentile (median and IQR were 0).

Discussion

This is the first prevalence study carried out in Sri Lanka on BK virus infection. Post renal transplant patients within two years of transplantation was selected for this study as they have the highest risk of adverse outcomes due to virus infection or reactivation. We detected a prevalence of 53.7% for overall viruria and 11% for overall viraemia. We detected seven patients with significant viruria and two patients with significant viraemia, that had reached presumptive PVAN cutoffs, needing further investigation and follow up [1].

The prevalence of an infection depends on the sensitivity and specificity of the assay. The assay we used, Altona Real-star® BK virus assay, was comparable with two other commercial assays and was even more sensitive than the other two assays [3].

Cross sectional studies done on BK virus prevalence are limited. A recent study from Sri Lanka [4] involving 15 post renal transplant patients with complications reported viruria in two patients but no viraemia. The median time after transplantation in this study was 2.6 years.

A recent study from Iran reported 41.8% viruria. A study from Kuwait reported 45% viruria and 26% viraemia in a group of patients with allograft dysfunction [5,6]. In our study the rate of viruria was somewhat higher but the rate of viraemia was comparable. The difference in rates may be due to the discrepancies in assay procedure and analytical sensitivity of the assay.

An Indian study reported incidence of viraemia of 42.9% one month post-transplant. An American follow up study reported that the highest rates of viruria (25.4%) and viraemia (13.7%) was at six months which then decreased at 12 months (20.3% and 8.6%, respectively) [7,8]. In this study the viral load cutoff to define viruria was ≥ 2500 copies/ml and viraemia ≥1000 copies/ml. The prevalence rates in our sample using the above viral loads were viruria 27.2% and viraemia 9.4%.

Thirty five patients in our study had elevated serum creatinine. This could be due to several reasons including BK virus associated nephropathy and graft rejection. In our sample the median serum creatinine levels and the proportion with elevated serum creatinine were higher in patients with significant viraemia. Similar association was not seen with significant viruria. These results were comparable to those of a study conducted among solid organ transplant patients which demonstrated that BK virus viruria or viraemia and mycophenolate are independent risk factors for impaired renal function [9]. However, another study conducted in Hong Kong had not observed any relationship between viruria or viraemia and serum creatinine levels [10].

We observed that the median urine viral load was very low (9.9 copies/ml) and viraemia was not detected in patients with normal serum creatinine. In the vast majority of these patients clinical BK virus associated nephropathy could be excluded. Thus patients with significant viral loads require more stringent follow up to detect BK virus associated nephropathy early.

There were several limitations in our study. The lack of consensus about pre-analytical processing of urine samples was one and this may have an impact on the rate of detection and overall viral load. While pelleted urine is recommended in one study, another larger study recommends whole urine as opposed to centrifuged urine pellet [11,12]. Further studies are required to resolve this issue. Serum creatinine levels were extracted from existing laboratory reports, therefore there would have been inter-laboratory variation.

Conclusion

Prevalence of BK virus viruria and viraemia were high among renal transplant patients within the first two years of transplantation. Significant viraemia was associated with raised serum creatinine levels. BK virus is undetectable in a significant proportion of post transplantation patients with normal serum creatinine. Prospective studies should be conducted to determine viruria and viraemia 'panic values'.

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Conflicts of Interest

Authors declare that there are no conflicts of interest.

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