Decoy Cell Viruria in Kidney Transplant Patients. Does it correlate with Renal Function?

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Abstract

Objective: BK virus (BKV) infection after kidney transplantation has been a topic of great interest in the recent decade. Prospective screening studies have revealed that BKVN is principally an early complication of renal transplantation occurring within the first post-transplant year in most cases. The aim of the present study was to observe the incidence of decoy cell viruria in renal transplant recipients. Furthermore, correlation of decoy cell viruria with graft function was assessed.

Methods: This analytic cross-sectional study was conducted in the Transplant Center of Alzahra Hospital, Isfahan, Iran between Jun 2014 and June 2015. Clinical screening for polyomavirus infection was done by means of urine cytological evaluation for decoy cells. Urine samples were analyzed in three steps including 2-4 months after transplantation, three and six months later.

Results: Thirty-three patients (22 male and 11 female) received kidney transplant from living donors. The average of patients' age was 41.9±12.83 (range: 20-63 years). Peritoneal and hemodialysis were used for 15.6% and 84.4% of recipients. The occurrence of decoy cell viruria at the time of enrollment, 3 and 6 months later was found in 18.2%, 10.7% and zero, respectively.

Conclusion: As urine cytology is easy to perform and of low cost, it is a useful tool for the investigation of active polyoma virus infection. Moreover, the findings advocate that the presence of decoy cells along with high creatinine is a better indicator of the virus presence.

Key words: BK Virus, Decoy Cell Viruria, Renal Transplantation, Renal Function

Introduction

BK virus (BKV) infection after kidney transplantation has been a topic of great interest in the recent decade. Human polyoma viruses are the members of the papova virus family which have a double strand DNA genome. The most identified species of this kind are BK-virus, JC-virus (JCV) and Simian-virus. BKV was first isolated from the urine of a renal transplant recipient with ureteric stenosis in 1971, but until 20 years later BKV was not recognized as a reason of interstitial nephritis and allograft failure in renal transplant patients. The preliminary infection may occur through fecal-oral transmission, respiratory tract and over the placenta. Also, they can be transmitted through organ transplantation. The vast majority of polyomavirus associated nephropathy (PVN) is triggered by the BKV, and the JCV is responsible for less than 3% of cases. (1)

BKV nephropathy (BKVN) which is involving 1-7% of renal transplant recipients, presented as a slow increase of serum creatinine. Prospective screening studies have revealed that BKVN is principally an early complication of renal transplantation occurring within the first post-transplant year in most cases. (2) Although the pathological view of tubulointerstitial nephritis can mimic rejection, the treatments for these two conditions are dissimilar: While dose reduction of immunosuppressant is the treatment of tubulointerstitial nephritis, treatment of rejection is by increase in immunosuppressant dose. (3)

As BKVN has restricted treatment options, the goal of screening is to facilitate primary diagnosis of patients when viruric or viremic, and to interfere before the development of overt nephropathy. After BK recurrence, the virus is first detectable in the urine, however, viremia develops after several weeks. Despite guidelines recommending quantitative polymerase chain reaction (PCR) for screening, urinary decoy cell detection is a potentially cost-effective alternative. (4) The aim of the present study was to observe the incidence of decoy cell viruria in renal transplant recipients. Furthermore, correlation of decoy cell viruria with graft function was assessed.

Methods and Materials

Recruiting patients

This analytic cross-sectional study was conducted in the Transplant Center of Alzahra hospital, Isfahan, Iran between Jun 2014 and June 2015. Ethical approval was attained from the local research ethics committee in school of medicine, Isfahan University of Isfahan before enrollment. (Approval code: IR.MUI.REC.1393.3030367, research project code: 393367) Informed written consent was obtained from all cases before recruiting in the study. Consecutive kidney transplant recipients from living donors who were older than 18 years were included. The inclusion criteria were to pass 1-4 months from transplantation. Patients who had a positive history of acute renal rejection or urothelial cancers were excluded. Also, patients were excluded from the study if they were unable to continue due to any causes. In all patients a comprehensive questionnaire including recipient demographic features, past drug history, concomitant diseases, type and duration of dialysis and time after transplant were recorded.

Laboratory tests

Clinical screening for polyomavirus infection was done by means of urine cytological evaluation for decoy cells. Urine samples were analyzed in three steps including 2-4 months after transplantation, three and six months later. Early in the morning the patient voided the urine collected in the bladder overnight; the next fresh urine sample was referred to cytology laboratory within 15 minutes of micturition; 0.5-1 mL of urine was processed by liquid based cytology. Slides were immediately fixed in 95% alcohol for Papanicolaou staining. Time interval between the day of transplantation and first appearance of decoy cells in the urine and period of decoy cell persistence in the urine were assessed. Also, the number of decoy cells was counted in each smear. Qualitative urine and blood PCR for BKV DNA performed for patients were positive for presence of decoy cells in their urine cytology. Moreover, urine analysis was performed for all patients. Urine cytology was performed at 3 and 6 months after the first evaluation. Simultaneously, in order to assess renal function, serum creatinine was measured three times. Since GFR is considered as a highly sensitive and specific scale for chronic renal failure, it was calculated by MDRD formula based on serum creatinine. Transplant kidney biopsy was performed considering medical indications approved by expert nephrologist (5).

Statistical analysis

All data were analyzed using the SPSS®23 statistical software package. Quantitative demographic characteristics are expressed as mean ± standard deviation (SD) and qualitative data are shown as a percentage. To compare means of two normally distributed data, the Student's t-test was used. For non-normally distributed data, the Mann-Whitney U-test was used. For comparisons of the correlations between the two groups, the chi-square and Fisher's exact tests were used. A p-value of <0.05 was considered statistically significant.

Results

Demographic data

Thirty-three patients (22 male and 11 female) received kidney transplant from living donors. The average of patients' age was 41.9±12.83 (range: 20-63 years). Peritoneal and hemodialysis were used for 15.6% and 84.4% of recipients. After transplantation, patients received prednisone, cyclosporine, and mycophenolate mofetil. The average of months of interval between transplantation and the first assessment was 2±0.9 months (range: 1-4). Demographic, clinical and para-clinical information of transplant recipients are revealed in Table 1.

Table 1: Demographic, Clinical & Paraclinical Information of Transplant Recipients Revealed

Patient demographic and lab data	Value
Age	41.9 ±12.83
Sex	
Male	66.7(%22)
Female	33.3 (%11)
Type of dialysis	
HD	81.8 (%27)
PD	15.5 (%5)
Non	3 (%1)
Duration on dialysis(month)	16.5 ±15.20
Months post transplantation	2.0 ±0.9
Last creatinine before sampling	1.31 ±0.24
Mena GFR	59.8±11.8
Cause of renal failure	
DM	24.2(%8)
HTN	21.2(%7)
GN	18.2(%6)
ADPKD	12.1(%4)
Others	24.2(%8)

Figure 1: Plots of creatinine (a) and GFR (b)in three consecutive samplings

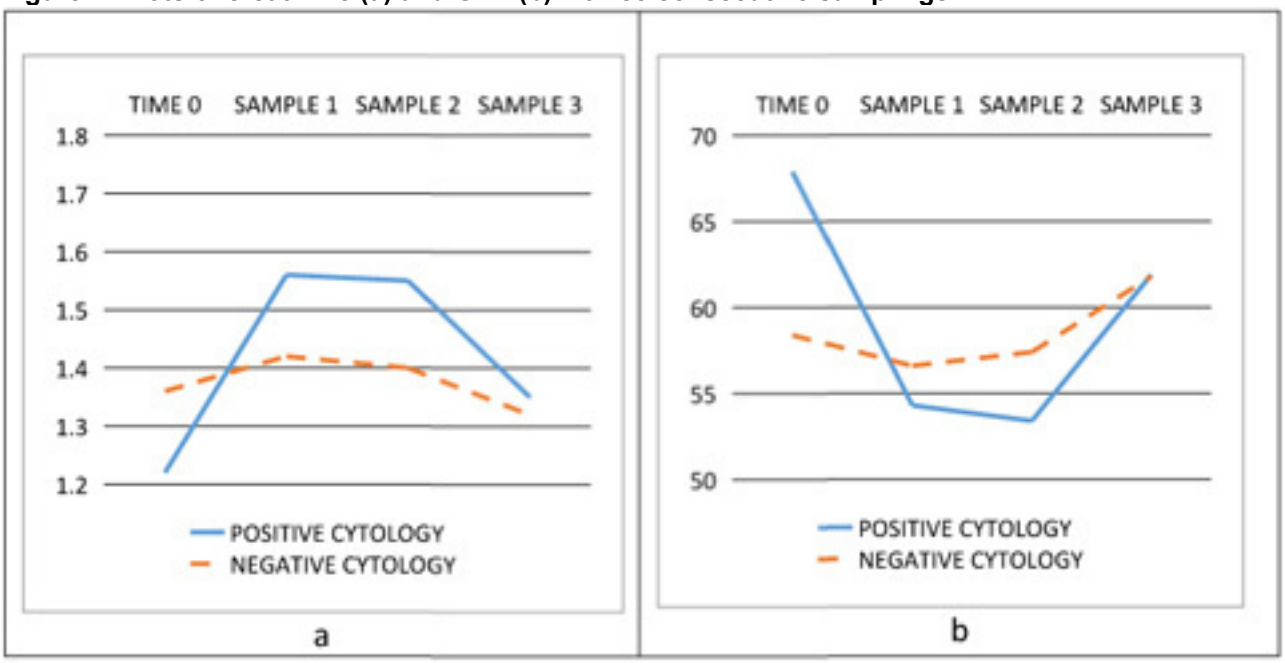
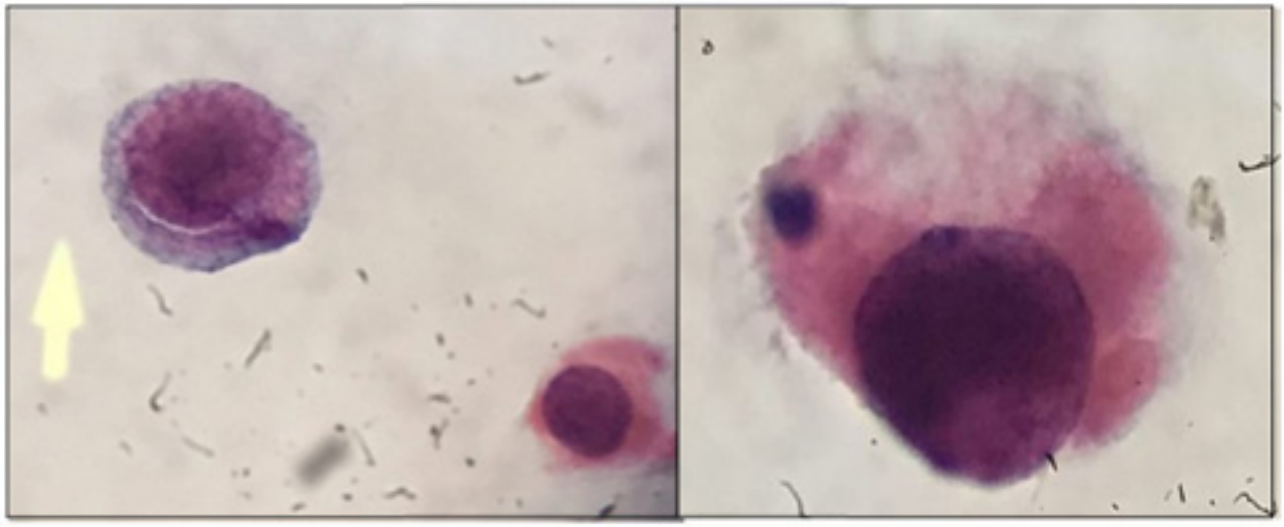


Table 2: Decoy Cell Per Smear

	1st step	2nd step	3rd step
Patient 1	4	6	0
Patient 2	15	0	0
Patient 3	3	3	0
Patient 4	13	12	0
Patient 5	3	0	0
Patient 6	1	0	0

Figure 2: Decoy cells with enlarged nucleus with a baso-philic intra-nuclear inclusion (papanicolaou stain *400)



Occurrence of decoy cell viruria

Urine decoy cells were assessed in three mentioned intervals (at the time of enrollment, 3 and 6 months later). Definite presence of decoy cell, was proved by qualitative PCR of urine in all cases with positive sample (Figure 1). The occurrence of decoy cell viruria at the time of enrollment, 3 and 6 month later was found in 18.2%, 10.7% and zero, respectively. One case with decoy cell viruria and positive for CMV and BK-PCR underwent renal biopsy and showed no viral changes. The number of decoy cells in each high power field is shown in Table 1.

Evaluation of renal function

Serum creatinine and urine analysis were used for the evaluation of renal function. The level of creatinine was 1.43±0.29 mg/dL, 1.39±0.24 mg/dL and 1.35±0.26 mg/dL in the three steps of the survey. Also, estimated GFR (eGFR) was calculated using the MDRD formula. In three steps of follow up the value of eGFR was 55.3±11.4 mls/min/1.73m2, 57.3±11.7 mls/min/1.73m2 and 61.8±14.3mls/min/1.73m2. The urinary WBC count was 8.0±10.4, 7.4±4.1 and 6.7±3.3 in three intervals. Moreover, the urinary count of RBC was 23.3±17.7, 9.2±3.4 and 2.1±1.6 respectively.

Correlation of decoy cell viruria and renal function

Independent t-test demonstrated that there is a significant difference between renal function and decoy cell viruria after 2 months of follow up. (P= 0.017) Moreover, the count of RBC was significantly lower in patients with decoy cell viruria (P= 0.001). After 5 months of follow up the level of creatinine was significantly higher in patients with decoy cell viruria (0.3±0.17 vs 0.2±0.04). Results of Spearman's rho test are demonstrated in Table 2. Regarding all of the 93 samples the level of creatinine was significantly higher in patients with decoy cel viruria. Additionally, there was no significant difference between occurrence of decoy cell viruria and count of WBC and RBC. In order to make a better correlation between decoy cell viruria and renal function, we divided patients into two groups, including GFR lower than 60 (group A) and larger and equal to 60 (group B). The average of patients' age in group A was significantly higher than group B (46.2±11.5 vs 36.2±12, P<0.001).

The GFR of groups A and B were 65±13 and 56±9 respectively and they were significantly different. Male patients were significantly more in group A rather than

females. 89% of patients with positive decoy cell viruria were in group A, while 60.2% of patients without decoy cell viruria were in this group (P=0.039). Since there was a significant correlation between post-transplantation GFR and age, sex and pre-transplantation GFR, we used logistic regression test to control their confounding effect. According to this, we concluded that there was a significant correlation between post-transplantation GFR and positivity for decoy cell viruria (OR=11.6; 95% CIs 1.12-120.04, p=0.02) (Figure 2).

Discussion

One of the leading causes of graft loss after kidney transplantation is polyomavirus. JC and BK virus infection is very prevalent in the first two years after transplant and might be monitored appropriately (6). Routine screening for BK has been shown to be effective in preventing allograft loss in patients with BK viruria or viremia. Reduction of immunosuppression remains the mainstay of BK nephropathy treatment and is the best studied intervention (7). The present study was conducted on thirty-three patients (22 males and 11 females) who received kidney transplantation from living donors. The average of patients' age was 41.9±12.83 (range: 20-63) years. In a similar study in Iran thirty-one patients (21 men and 10 women) received kidney transplant from living donors. In this study, the average of patients' age was 38.3±12.8 (rage: 17-59) years (8). Urine cytology is a safe, noninvasive and sensitive tool for the evaluation and follow-up of renal transplant recipients and can be used as prospective screening for BKV allograft nephropathy (9). In BKV nephropathy, the finding of urinary decoy cells showed a 100% sensitivity, 84% specificity, 100% negative predictive value and 6% positive predictive value (10). In the first step of follow up of our study, the presence of decoy cells was 18.2% in kidney transplant recipients, while another report demonstrated the presence of decoy cells in 37.5% of patients (8). In this study, the occurrence of decoy cell viruria at the time of enrollment, and 3 and 6 months later was 18.2%, 10.7% and zero, respectively. Moreover, in another study the prevalence of polyoma virus infections increased with increasing time after transplantation (11), which is similar to the study by Liu et al. (12) Another same study revealed that Urinary decoy cell shedding was detected in 26.2% of 286 cases. BKV viruria was observed in 22.1% of 938 cases and BKV viremia in 5.2% of 1,029 cases. (13) One study in 2007, presented that significant polyoma viruses viruria is common following renal transplantation with onset usually within the first 3 months. Viruria is associated with worse graft function at 3 and 6 months. The time between urine positivity and clinical polyoma virus nephropathy is short. More frequent early urine screening would be required to achieve clinical benefit. In another study, the incidences of viruria and viremia at 1 year were 35% and 11.5%, respectively, compared with 17% and 3% at a time of 49 months post-transplantation.(10) Although managing a BKV infection includes reducing immunosuppression alone or combined with antiviral therapy, such as cidofovir or leflunomide, only an early diagnosis and reduction of immunosuppression reliably improve graft survival. (13)

The present study also revealed that the level of plasma creatinine was significantly higher in patients with decoy cell viruria. This correlation is similar to another study demonstrating that patients with BK-virus nephropathy had high serum creatinine that mimicked either tubular necrosis or rejection (14). The same study in 2006, suggested that the presence of decoy cells along with high creatinine is a better indicator of the virus presence. According to this study, there was a significant correlation between post-transplantation GFR and positivity for decoy cell viruria. Despite a low positive predictive value of decoy cells in urine, its absence has a negative predictive value of 100%, because almost all of those patients who did not have decoy cells had normal renal function.

Conclusion

In conclusion, our findings suggest that considering the risk of graft loss due to polyoma virus infection, routine urine cytology might be used as a screening method for the detection of polyoma virus infection. As urine cytology is easy to perform and of low cost, it is a useful tool for the investigation of active polyoma virus infection. Moreover, the findings advocate that the presence of decoy cells along with high creatinine is a better indicator of the virus presence (15).

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